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LIFE SPAN AND MORPHOLOGY OF FIRE BLIGHT BACTERIA AS INFLUENCED BY RELATIVE HUMIDITY, TEMPERATURE, AND NUTRITION[1]

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INTRODUCTION

The problem concerning the sources of inoculum for the earliest or primary fire blight on apple and pear trees in the Ozarks of Arkansas remains obscure. Unlike more northern and western fruit-growing areas, where excellent evidence has been found for the overwintering of the pathogen in formerly blighted limbs and twigs and its dissemination from such hold-over blight to blossoms and twigs, no such evidence has been found in the Ozarks (*11, 13, 14*).[2] This should occasion no surprise. The marked differences in climatic conditions, in length of growing season, and in varieties grown must undoubtedly have some influence on the variation of the life of the pathogen. The relative scarcity or absence of fire blight west of the Cascades is well known, in contrast to its prevalence in fruit-growing areas east of this mountain range, and as Heald (*7*) has noted:

> * * * sufficient time has elapsed since its arrival in the coast country for it to have become prevalent in western Washington, hence it seems possible that climatic factors are responsible for its absence from this section.

In an effort to discover the sources of inoculum for early spring infections in the Ozark region, various lines of investigation have been pursued, including the possible overwintering of fire blight bacteria within beehives (*13*) and the overwintering of bacterial exudate either on parts of the trees above ground or in the soil (*16*). However, none of the evidence deduced from these studies is conclusive (*13, 16*). The problem is still unsolved, not only for the Ozarks of Arkansas but for the Southern States as a whole, although the use of open-blossom sprays by the writer (*14*) has been based on the assumption that the first spring blight is occasioned mainly by the dissemination of the bacteria by blossom-visiting insects, especially bees.

It is clear, however, that without knowledge of the influence exerted by various combinations of climatic and nutritional factors on the life of this parasite, conclusions relative to the longevity and prevalence of bacteria drawn from such tests as artificial contamination of beehives, attempted isolations from infested hives, and isolations from insect bodies, soil, or diseased tree parts may have very restricted significance and at best be applicable only to local or purely artificial conditions. To gain more definite information on the influence of

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[2] Reference is made by number (italic) to Literature Cited, p. 257.

important climatic factors and to a limited extent of nutritional factors on the life of the organism, investigations reported here were undertaken. A special effort was made to study the behavior of the bacteria in naturally occurring exudate rather than their behavior in pure, artificial cultures, although the latter were used for comparison.

REVIEW OF PREVIOUS WORK

Since a review of the older literature pertaining to the influence of dryness on the longevity of the fire blight organism, *Erwinia amylovora* (Burrill) Winslow et al. (*Bacillus amylovorus*), has been presented previously (*16*), there is no need of duplicating it here. In that paper evidence was presented to show that the bacteria in the form of exudate derived from blighted tissues are very much less sensitive to dry air than had generally been supposed; and that at a controlled relative humidity approaching zero they were found to be alive at the end of a year under laboratory conditions of fluctuating temperatures and reduced light. Likewise, it was reported that exudate kept outdoors in corked vials, under uncontrolled light, temperature, and humidity conditions, yielded dead bacteria in short order, and that the organisms in the form of hardened exudate left in place on a cankered pear trunk exposed to direct sunlight also died within a few days.

Good evidence has very recently been presented by Parker (*10*) confirming the findings relative to the longevity of *Erwinia amylovora* under dry conditions, and adding to our knowledge concerning the combined influence of temperature and humidity. Using temperatures of 31°, 52°, and 70° F. and both very dry conditions, maintained by the use of calcium chloride desiccators, and more moist conditions in open dishes, he found that bacteria derived from exudate lived at least 52 days, the last day of testing, at 31° and 52°, and at least 45 days at 70°, all under dry conditions, while under more moist conditions in open dishes they were shorter lived.

Ark (*1*) has also reported very recently on studies of fire blight bacteria which included the influence of dryness on the life of the bacteria. First he reports very briefly that he "was able to isolate viable bacteria from bacterial exudate kept in a vial in a dry laboratory closet for 2 years and 10 months." Whether these bacteria were tested for pathogenicity is not stated, and one wonders what tests were used to determine if the isolated bacteria actually represented *Erwinia amylovora*. However, he performed two additional experiments involving the influence of dryness, though both were primarily concerned with discovering differences in reaction of different isolates. Using four isolates derived from unlike hosts and from diverse parts of the United States, he grew them in beef-extract agar, suspended the growths in a saline solution, and tested for the influence of dryness by smearing a drop of the bacterial suspensions on cover glasses maintained at different degrees of dryness. The bacteria survived from 18 to 36 hours, the latter period representing the longest utilized. "The degree of humidity seemed to be of no significance as far as the longevity of *E. amylovora* is concerned in this experiment," he concluded. In a second experiment, which also involved artificial culture media, gauze strips suspended in broth cultures were removed after 48 hours of growth, the strips hung in large sterile test tubes, and pieces cut off and cultured from time to time. Two isolates were

found to be nonviable after 6 days, a third after 4 days, and the fourth after 10 days. He reported:

> Both of the experiments were repeated and in each case the same results were obtained. Thus, it is safe to conclude that different degrees of susceptibility to dryness exist among isolates of *Erwinia amylovora* * * *.

Ark makes no comment of the amazing discrepancy in longevity under dry conditions shown in his test of viability of bacterial exudate compared with his tests of pure cultures.

So far as studies on the influence of temperature are concerned, most of these have in the past involved pure cultures, usually maintained in beef-broth or synthetic media in which the standardized procedure of determining maximum, optimum, and minimum temperatures for growth was followed and little attention paid to the life span of the bacteria at the different temperatures. With the exception of the very recent paper by Ark (*1*), these former temperature studies have been reviewed *(14, p. 4)*. Ark followed the same procedure used by previous investigators and obtained comparable results.

With reference to nutritional factors influencing the longevity of this organism, the writer has no knowledge of earlier work. At times it has been assumed that fire blight bacteria grown in beef-broth or nutrient agar will be comparable, morphologically and physiologically, to those growing within apple or pear tissues, and such tests as applying beef-broth or agar cultures to beehives followed by attempts to reisolate the parasite after varying intervals of time from the hive have been used with the assumption that the data obtained would be comparable to those resulting from the transfer of bacteria from a pear or apple blossom to the hive by a honeybee. That differences in nutrition—as exemplified by growth in beef-broth or nutrient agar compared with bacterial growth in natural host tissues, or in nectar—might conceivably yield wholly unlike results in physiological response as well as in morphology had not, to the writer's knowledge, been entertained.

Since part of the investigations herein described concerns differences in morphological response, particularly that which deals with the presence or absence of a capsule, sheath, or slime formation, several investigators report the absence of capsules for this species of bacteria (*5, 8, 19*).

MATERIALS AND METHODS

The bacterial exudate utilized consisted of four collections. Lot 1 was made up of the exudate placed over concentrated sulphuric acid on June 11, 1934, used in earlier work (*16*) and continued in these investigations.

Lot 2 consisted of exudate gathered on May 21 and 28, 1935, from freshly blighted shoots and blossom clusters of Jonathan apple trees located in a commercial orchard near Rogers, Ark. This material was kept in a refrigerator overnight and placed the next day in small, paraffin-covered paper baskets suspended in 200-cc bottles over concentrated sulphuric acid having a specific gravity of 1.835. Most of this exudate when gathered was in the form of small, milky, or light-brown droplets which had not fully hardened and were still quite viscid. In many instances it was found necessary to scrape the epidermal hairs of the young twig, petiole, or pedicel, so that the droplets of exudate as placed in the bottles were often enmeshed in these hairs. No attempt was made to sterilize the bottles or to remove the

exudate from the blighted material under aseptic conditions, since one of the objectives of these investigations was to determine the longevity of fire blight bacteria in the presence of naturally occurring, competing, or antagonistic micro-organisms that might be present in the orchard.

On June 21, after the material had been kept under the same conditions of relative humidity, temperature, and light for a period of 3 to 4 weeks, and had reached the same degree of dryness, as measured by the specific gravity of the sulphuric acid, it was divided and treated as follows: 22 divisions were made to be kept under as many combinations of relative humidity and temperature. To obtain different relative humidities, various concentrations of sulphuric acid-water mixtures were made, the physical table presented by Wilson (*21*) being used as the guide. Specific-gravity determinations used as a check on the theoretical computations indicated that the following relative humidities were obtained: 0, 9.5, 21, and 45 percent. The amount of sulphuric acid or sulphuric acid-water mixture utilized, the placement of the open baskets, the size of the bottles used, the sealing of the rubber stoppers with melted paraffin, all followed the same methods described in the former article (*16*). For the exudate kept at approximately zero relative humidity, six bottles were used, one for each of the following temperatures: 16°, 25°, 30°, 35°, and 40° C., and outdoors (fluctuating temperature). The last bottle was tied in a wire basket and suspended from an upper limb of a large apple tree. The other three relative humidities, in combination with the different temperatures noted, were utilized as in the zero relative humidity, except that insufficiency of exudate did not permit using the outdoor temperatures for all the relative humidities.

Lot 3 of exudate was gathered on May 19, 1936, from freshly blighted Bartlett pear shoots. It was immediately placed over concentrated sulphuric acid, allowed to harden thoroughly, and on June 15, 1936, divided into two parts, one placed over an acid-water mixture yielding an approximate relative humidity of 45 percent, and the other over concentrated sulphuric acid, specific gravity 1.835. They were then transferred outdoors into United States Weather Bureau-type housing.

Lot 4 of exudate was also gathered from freshly blighted Barlett pear shoots on July 30 and August 3 and 14, 1936. It was placed over concentrated sulphuric acid on each date of collection and kept at laboratory temperatures until September 4, 1936. On that date the thoroughly hardened droplets were divided into three parts, placed in bottles containing sulphuric acid-water mixtures which yielded relative humidities of approximately 50, 75, and 90 percent, and were kept at 25° C. for 5 days. They were then taken outdoors and placed in the United States Weather Bureau-type housing.

As previously noted, the bacterial exudate in the different lots was first placed over concentrated sulphuric acid, so that all of it would be brought to approximately the same degree of dryness. It seemed essential to have all the material as uniform as possible relative to the amount of moisture present, prior to its use for experimental purposes.

In order to study the influence of dry air and different temperatures on *Erwinia amylovora* when present within blighted host tissues, freshly diseased twigs and leaf petioles of Jonathan apples were gathered on May 21, 1935, in the same orchard from which exudate was obtained. The day after the material was gathered it was cut

into lengths varying approximately from one-fourth to three-eighths inch, so that it could be accommodated in the small baskets kept over the acid. An effort was made to use only fresh infections; all material that showed signs of hardening of the tissues and last stages of blight development were rejected. However, subsequent tests revealed that some of the material, in addition to harboring *E. amylovora*, was also the seat of nonpathogenic, yellow bacteria—rather common followers of fire blight bacteria within diseased tissues (*12*)—and hence was not representative of strictly fresh infections. Fortunately the number of pieces showing such secondary invasion was not large.

This diseased material was divided into two lots, both placed over concentrated acid, but one was kept in a bottle placed outdoors and suspended from an apple limb in the same manner as described for the bacterial exudate kept outdoors, and the other was kept under laboratory conditions.

The pure cultures of *Erwinia amylovora* used for these tests were made up as follows: A recent isolate which had proved to be highly virulent in artificial infection was replated on nutrient dextrose agar, and after 48 hours incubation, a single vigorous colony was transferred in the form of smears on a plate of the same medium used previously. After 48 hours, a second set of smears was made, again on the same medium, and also transferred to tubes of nutrient dextrose broth. After 48 hours' growth, the smears, together with the underlying medium, were cut into blocks approximately 1 cm square, and immediately placed to dry in open baskets over concentrated acid in corked and sealed bottles. At the same time, one of the tubes of broth with 48 hours' growth was employed for testing the pathogenicity of the isolate. Several healthy, succulent Bartlett pear shoots were injected with the bacterial suspension, and within several days all inoculated shoots showed characteristic blight symptoms, while uninoculated controls remained free.

At intervals following the placement of the different materials in the various relative humidities and temperatures, parts were removed and treated as follows: A droplet of exudate was placed in approximately 0.2 to 0.5 cc of sterile distilled water, resulting in a very heavy bacterial suspension, which was injected with a sterilized hypodermic syringe into healthy succulent Bartlett pear shoots; or cultures were plated out and single-colony transfers used for injecting pear shoots. In the first few series of trials injections were made from single-colony transfers but this was found to be unnecessary because it was discovered that whenever viable *Erwinia amylovora* were present in sufficient numbers to induce infections, direct injections of the original exudate suspended in water were just as reliable in testing the presence of live bacteria as the more cumbersome plating-out, single-colony transfer, and final injection method. Furthermore, in plating out, there is always the danger of losing any relatively slow-growing pathogen. In both cases the final proof for the viability and infectivity of the bacteria was based on the presence or absence of typical fire blight symptoms a few days following the artificial inoculations and on the presence or absence of *E. amylovora* in the infected tissues. If no fresh exudate appeared on the artificially infected tissue, the latter was surface-sterilized, washed in sterile water, and used for a series of poured dilution plates. When typical colonies appeared, single ones were re-isolated and again used for inoculation.

To determine the presence of viable bacteria within diseased tissues kept under the conditions indicated previously, several pieces were removed at different times, soaked and mashed in sterile water, and then used in a similar manner for testing the presence of viable bacteria as in exudate. Likewise, to test the viability of bacteria on the agar sheets (pure cultures), several of these were removed from the bottles at any one test, allowed to form heavy bacterial suspensions, and injected into healthy succulent Bartlett pear shoots.

All inoculations were made on trees growing in a greenhouse, and a sufficient number of uninoculated control shoots were at hand for comparative purposes as each test was undertaken. No infections occurred on the controls during any of the trials.

To determine the viability of fire blight bacteria in honeycomb, commercial honey encased in a wooden frame with the comb in place was obtained. On October 3, 1936, minute droplets of fresh, sticky exudate obtained from blighted Bartlett pear shoots, were introduced into the honey cells, care being taken to see that each droplet was immersed in the honey. Likewise, small, 3- to 5-mm pieces of freshly blighted leaf petioles were immersed in other honey cells on the same date in order to determine any difference in reaction between the bacteria present in exposed exudate and those within blighted tissues.

EXPERIMENTAL RESULTS

Table 1 presents the results of these tests. The following additional data pertain to the materials and experimental conditions shown in table 1.

With reference to the four lots of exudate kept at the temperature recorded at 16° C., temperatures were read and recorded three times each week day—a procedure followed for all the different recorded temperatures shown in table 1. The readings for the 16° temperature showed but slight variation, the lowest being 15° and the highest 18°, the average or mean temperature being 16° and with only occasional variation from that figure. The material was kept in an incubator, especially useful for controlled low temperatures.

The different relative humidities were checked by means of hydrometer readings shortly after the last tests were made for viability and infectivity. These readings were interpolated between those presented in Stevens' table (*18*) in which various specific gravities of sulphuric acid-water mixtures are recorded in terms of relative humidities. The results of these comparative readings of the initial relative humidities and those obtained more than a year later are presented in table 2.

The temperature of 25° C. recorded in table 1 is only roughly accurate. The incubator utilized for this temperature held a fairly constant temperature when the laboratory temperature did not exceed 25°, but gave no good control at high laboratory temperatures. Daily readings throughout the period of investigation show that during the summer and early fall the readings fluctuated from 25° to 32°, although throughout the remainder of the year the temperature of 25° was fairly well maintained.

The 30° C. temperature shown in table 1 was held fairly constant in an incubator throughout the period of testing, with only slight fluctuations, which were usually of short duration. The fluctuations varied mainly from 28° to 32°, with one reading recorded at 36°.

The 35° C. storage temperature shown in table 1 was fairly well maintained in an incubator. The fluctuations varied from 32.5° to 40°, these being extremes. Most of the recorded fluctuations were between 34° and 36°.

TABLE 1.—*Viability and infectivity of Erwinia amylovora kept at various relative humidities and temperatures in the form of exudate, within diseased tissues, and as pure cultures*

Type of material	Approximate relative humidity	Temperature	Days viable and infectious [1]	Nonviable at any time tested (dates of tests)
	Percent	*° C.*	*Number*	
Exudate	0	16	[2] 320	
	9.5	16	[2] 571	
	21.0	16	[2] 571	
	45.0	16	[4] 400	
	0	25	[2] 412	
	9.5	25	[2] 412	
	21.0	25	[2] 421	
	45.0	25	[5]	
	0	30	[2] 408	
	9.5	30	[2] 674	
	21.0	30	[2] 408	
	45.0	30	----------	Jan. 22, Feb. 11, May 4, July 29, July 31, Aug. 7 and 13, 1936.
	0	35	[2] 320	
	9.5	35	[2] 426	
	21.0	35	[6] 320	
	45.0	35	----------	Jan. 14, Feb. 11, May 4, Aug. 18 and 26, 1936; Jan. 16, 1937.
	0	[7]	[2] 362	
	45.0	[7]	[2] 244	
	0	40	[8] 400	
	9.5	40	[5]	
	21.0	40	[9] 321	
	45.0	40	----------	Dec. 18, 1935; Feb. 11, May 5, Aug. 21 and 27, 1936.
	0	[10]	[11] 187	
	[12]	[10]	----------	Dec. 27, 1935; Jan. 14, May 25, 1936.
Diseased tissue	0	[12]	[2] 351	
Exudate	0	[13]	[14] 268	
	50.0	[13]	[2] 276	
	75.0	[13]	[10] 55	
	90.0	[13]	[17] 7	
Agar culture	0	[13]	----------	Jan. 15, Feb. 12, 1936.
Exudate	0	[13]	[18] 610	
Exudate in honey	[12]	[13]	[19] 22	
Diseased tissue in honeycomb.	[12]	[13]	[20] 121	

[1] This consists of the length of time in which viable and infectious bacteria were found in the different materials kept under designated humidity and temperature conditions, figured from the time that the material was first placed at the allotted humidity and temperature to the last date utilized for testing.
[2] No material remained for testing after the number of days indicated.
[3] No tests were made beyond the number of days indicated.
[4] Material not tested after 400 days.
[5] The material included in this test was accidentally destroyed early in the work.
[6] Tests made at the end of 426 and 444 days showed no viability.
[7] Outdoors (weather housing).
[8] Tests made at the end of 428 and 431 days showed no viability.
[9] Tests made at the end of 341 days and all later ones showed no viability.
[10] Outdoors (apple tree).
[11] Tests made at the end of 237 days and beyond showed no viability.
[12] Fluctuating.
[13] Laboratory.
[14] Outdoors.
[15] Material was inadvertently destroyed shortly after the last date of testing.
[16] Tests made at the end of 124 days and beyond showed no viability.
[17] Tests made at the end of 22 days and beyond showed no viability. The exudate at this constantly high relative humidity became coated with saprophytic molds within a few days time.
[18] Material kept over concentrated acid from June 12, 1934, to Feb. 11, 1936, when it was exhausted.
[19] Tests made at the end of 92 days and beyond showed no viability.
[20] Tests made at the end of 256 days showed no viability.

The 40° C. recorded in table 1 was also held fairly constant in an incubator, the usual fluctuations varying from 39° to 41°. Extreme fluctuations occurred on 4 different days, on 3 days when the temperature reached 44° and on another when it dropped to 28° (but restored in a few hours to the desired temperature).

As noted in table 1, the number of days in which the various materials showed the presence of viable and infectious bacteria represent the elapsed time from the placement of the materials under the designated controlled conditions to the date of the last test. This does not represent the ultimate length of time that may be involved in the longevity of *Erwinia amylovora* under those conditions in which the bacteria were found to be viable in the last test made.

Although an effort was made to use approximately equal quantities of inoculum for each viability test of exudate, in practice this was found to be impossible because of the variation in size of the droplets of exudate and the presence of epidermal hairs of host tissues to which many of the droplets were attached.

The exudate kept under fluctuating laboratory temperatures and under approximately zero relative humidity represents a continuation of the tests of the same lot of material presented in a former article (*16*). At the end of 610 days, the last date of testing, the material was exhausted.

TABLE 2.—*Comparison between initial relative humidities and those present more than a year later at different temperatures*

Approximate initial relative humidity	Incubation temperature	Specific gravity a year later	Temperature at specific-gravity determinations	Corrections for temperature in specific gravity	Approximate relative humidity more than a year later
Percent	° *C.*		° *C.*		*Percent*
0	40	1.815	36	1.835	0
21.0	40	1.460	36	1.476	21.5
45.0	40	1.350	36	1.365	45.0
0	35	1.815	37	1.835	0
9.5	35	1.570	36	1.586	9.5
21.0	35	1.460	36	1.476	21.5
45.0	35	1.350	36	1.365	45.0
0	30	1.815	33	1.833	0
9.5	30	1.570	33	1.584	9.6
21.0	30	1.460	33	1.474	21.5
45.0	30	1.350	33	1.363	45.0
0	25	1.815	32	1.832	0
9.5	25	1.565	31	1.578	9.6
21.0	25	1.460	31	1.472	22.7
0	16	1.820	23	1.831	0
9.5	16	1.580	21	1.584	9.5
21.0	16	1.470	21	1.474	21.5
45.0	16	1.355	21	1.358	45.2

It is to be noted in table 2 that the relative humidities obtained more than a year after they had been made up show slight difference or none at all from the initial ones. This is to be expected since the amount of moisture present in the droplets of exudate, or even in the blocks of agar cultures or host tissues, must have exercised an insignificant effect on the relative humidity in any one bottle because of the comparatively large volume of concentrated sulphuric acid or mixtures of this acid and water present. Likewise, the use of rubber stoppers and the immediate sealing with hot paraffin after material was removed for any one test may be expected to prevent any marked influence of air humidities on those present in the bottles. The breaking of the paraffin seal, the removal of material, and the recorking and resealing were conducted as quickly as possible. At all tests these were performed in a small, dry, completely enclosed transfer chamber.

LONGEVITY OF BACTERIA IN FREE EXUDATE

Evaluating and interpreting the results shown in the tables, first with reference to the exudate kept under controlled temperatures and under various relative humidities, one notes that at 16° C. the exudates at the four different relative humidities still yielded viable and infectious bacteria more than a year after the initiation of the tests. This is in contrast with the exudates kept at the higher temperatures in which the bacteria were found to be dead in the first tests at a relative humidity of 45 percent, while at the lower relative humidities, from approximately 0 to 21 percent, the bacteria were viable at all the temperatures utilized, including that of 40° C. for long periods. Obviously, relatively high temperatures, unless accompanied by moderate or high relative humidities, do not seem to possess any marked germicidal action against the fire blight organism when in the form of natural exudate. In other words, so far as temperature is concerned, *Erwinia amylovora* can live for long periods as exudate under very diverse temperatures, but is relatively short-lived at moderate or high temperatures when the relative humidity is moderate or high. In the presence of fairly low temperatures, relative humidity does not appear to be such a limiting factor, at least not under uniform conditions of temperature and humidity.

The actual length of life of fire blight bacteria at an approximate relative humidity of 45 percent and at temperatures of 25° to 40° C. was not determined. They were nonviable at the first and at all the subsequent tests, but the first test at 40°, owing to shortage of material, was not conducted until 181 days after the material was first placed under the controlled conditions, and at 35° and 30° not until 208 and 216 days, respectively.

With reference to the exudate kept under fluctuating temperature conditions, it is to be noted in table 1 that there were 10 different types of treatment: (1) Exudate kept at approximately zero relative humidity under outdoor conditions, exposed to sunlight; (2) similar to the first but in the shade offered by Weather Bureau-type housing; (3, 4, 5) similar to the second but kept at relative humidities of 9.5, 21, and 45 percent respectively; (6) exudate kept outdoors under fluctuating conditions of relative humidity and temperature, that is, without any effort to control the relative humidity and temperature; (7) exudate kept at approximately zero relative humidity but under fluctuating laboratory temperatures; (8) exudate kept outdoors at approximately 50 percent relative humidity; (9) exudate kept outdoors at approximately 75 percent relative humidity; and (10) exudate kept outdoors at approximately 90 percent relative humidity. As noted previously, the last three were maintained in a Weather Bureau-type housing. Owing to the fact that it seemed desirable for comparative purposes to place the material in glass bottles comparable to those used for the other tests, an error was introduced in two of the tests which throws considerable doubt on their validity. It was found that the temperature within the bottles in the presence of direct sunlight was such that in one of them, that used for fluctuating humidity and temperature, the heat and probably moisture, was sufficient to cause the individual droplets to run together while in the other the droplets turned jet black and gradually seemed to have lost much of their weight. While the bacteria were found to be viable and infectious at the end of 187 days at approximately zero relative

humidity under such outdoor conditions, they were nonviable at the end of 237 days; and the bacteria kept under both uncontrolled temperature and humidity conditions showed no viability in the first and in the subsequent tests. However, when the exudate kept at approximately zero relative humidity was placed outdoors in a Weather Bureau housing, the exudate was found to contain viable and infectious bacteria up to the last date of testing, a period of 362 days, or practically a year. Likewise, it is particularly worth noting that while the bacteria kept outdoors in Weather Bureau-type housing at high relative humidities died in short periods of time, that kept at 50 percent relative humidity showed viable and infectious bacteria throughout the whole period of testing, 276 days, or over 9 months.

The tests for viability and infectivity of the bacteria present in exudate kept under fluctuating laboratory temperatures and at approximately zero relative humidity clearly indicate that under such conditions the bacteria remain alive and infectious for remarkably long periods. It is obvious that the 610 days, shown in table 1, in which the bacteria remained viable, is not a complete picture, for the material was exhausted at that time. There are no data to indicate the actual span of life of *Erwinia amylovora* under such conditions, though it may be surmised that it is probably considerably longer than that given.

LONGEVITY OF BACTERIA IN AGAR CULTURES

Contrasted with the long life of bacteria in the form of natural exudate under fluctuating laboratory conditions and at approximately zero relative humidity, it is of special interest to note the short life of bacteria when in the form of pure nutrient-agar cultures under the same conditions. The first test, made 205 days after the agar smears had been placed over the concentrated acid, showed that the bacteria were dead, and a duplication of the test yielded the same result. The actual life span, evidently very short, of *Erwinia amylovora* in the form of such pure cultures, was not determined. If one may judge by the behavior of agar slants of this species, the bacteria rarely remain alive more than a week or two under such conditions. This marked difference in longevity as agar cultures compared with natural exudate suggested a study of the morphology of the bacteria when grown as agar slant cultures or in beef broth and as found in exudate.

LONGEVITY OF BACTERIA IN BLIGHTED TISSUE AND IN HONEY

The longevity of fire blight bacteria when present within blighted host tissues and kept at approximately zero relative humidity, both under fluctuating laboratory temperatures and under fluctuating outdoor temperatures, is apparently comparable to the longevity of the bacteria when in the form of exudate. At the end of 351 days, they were still viable when kept under laboratory temperature conditions within diseased host tissues, and also at the end of 268 days, under outdoor temperature conditions. Unfortunately, the latter material was accidentally destroyed in the field shortly after this period. It is worth noting that some of the pieces of blighted host tissues, when mashed in sterile water and used for a series of poured dilution plates, yielded almost pure cultures of a nonpathogenic yellow organism, with very few or no colonies of the whitish fire blight bacteria appearing in a series of 10 or more plates. Likewise, when a

water suspension made from such pieces was injected into healthy Bartlett pear shoots, no infections were obtained. It was found, however, that if a sufficient number of pieces of host tissues were used for obtaining heavy bacterial suspensions, and injected directly into healthy pear shoots, there was no difficulty in proving the presence of viable and infectious fire blight bacteria, even though the nonpathogenic, yellow bacterium was present. This is in line with former investigations (*12*) in which it was found that measured mixtures of these two different species of bacteria still permitted infections to take place in artificial inoculations, provided the mixture was not permitted to stand longer than 4 hours prior to the inoculations. However, if such a mixture, made up in the proportion of twice as many fire blight bacteria as the yellow saprophytic bacteria, is permitted to stand for 8 hours in nutrient broth, the former is completely destroyed at the end of that time. Obviously the use of blighted host tissues for determining the presence of viable *Erwinia amylovora* must be used with these facts in mind. Likewise, it is clear that if one introduces nutrient media containing pure cultures of fire blight bacteria into beehives or into any other open receptacle subject to contamination by common saprophytes, the fire blight parasite will be in competition with other organisms, many of which are far more rapid growers and can exercise deleterious effects on the parasite. The experiments on supposedly freshly infected tissues subject to invasion by secondary or saprophytic micro-organisms, suggest that unless a clear distinction is made between the capacity of this organism to live under conditions not subject to the activity of other micro-organisms and its capacity to live in an environment that is subject to the growth of other micro-organisms, any investigation seeking to determine the behavior of this parasite will yield conflicting or questionable results.

It is especially worth noting that the bacteria in the form of minute droplets of exudate are capable of living at least 22 days immersed in honey under such outdoor conditions as afforded by Weather Bureau housing. Under the same conditions, bits of diseased host tissues were found to yield live bacteria up to 121 days; at the end of 256 days they were no longer viable.

MORPHOLOGICAL STUDIES OF SLIMY ENVELOPES AS FOUND IN ARTIFICIAL CULTURES AND IN NATURAL EXUDATE

As indicated in a previous publication (*15*), there is a striking difference in the viscidity of this organism as it is found under pure-culture conditions compared with natural exudate. As fresh exudate issuing from infected tissues, it is so viscid that when touched with a needle it strings out in long, thin threads, clearly suggestive of a sticky or gummy slime gluing the bacteria to each other; grown in pure cultures in beef broth or nutrient agar, or in any other standard culture medium, it rarely shows such striking viscidity. This in turn signifies either that the enveloping membranes, slime, or capsules produced under one set of conditions are quite different, physiologically and morphologically, from those produced under the other set of conditions, or that the use of artificial media suffused with water dissolves or changes this slimy matter.

To gain more definite knowledge on this subject, the bacteria as obtained under the two different conditions were subjected to various

stains particularly applicable for showing slimy envelopes, and also studied under the microscope in a living condition, without the use of stains.

When a fresh, milky drop of exudate is touched with a sterile needle and the exudate drawn out into very fine threads approximately 10μ in diameter, these threads dry rapidly under ordinary laboratory conditions. They are readily cut into short lengths and can be placed on a glass microscope slide shielded by a cover glass for study of the bacteria in a living condition. Such studies make possible the observation of individual bacteria present in exudate without recourse to any medium such as water, alcohol, or stains that may produce artifacts.

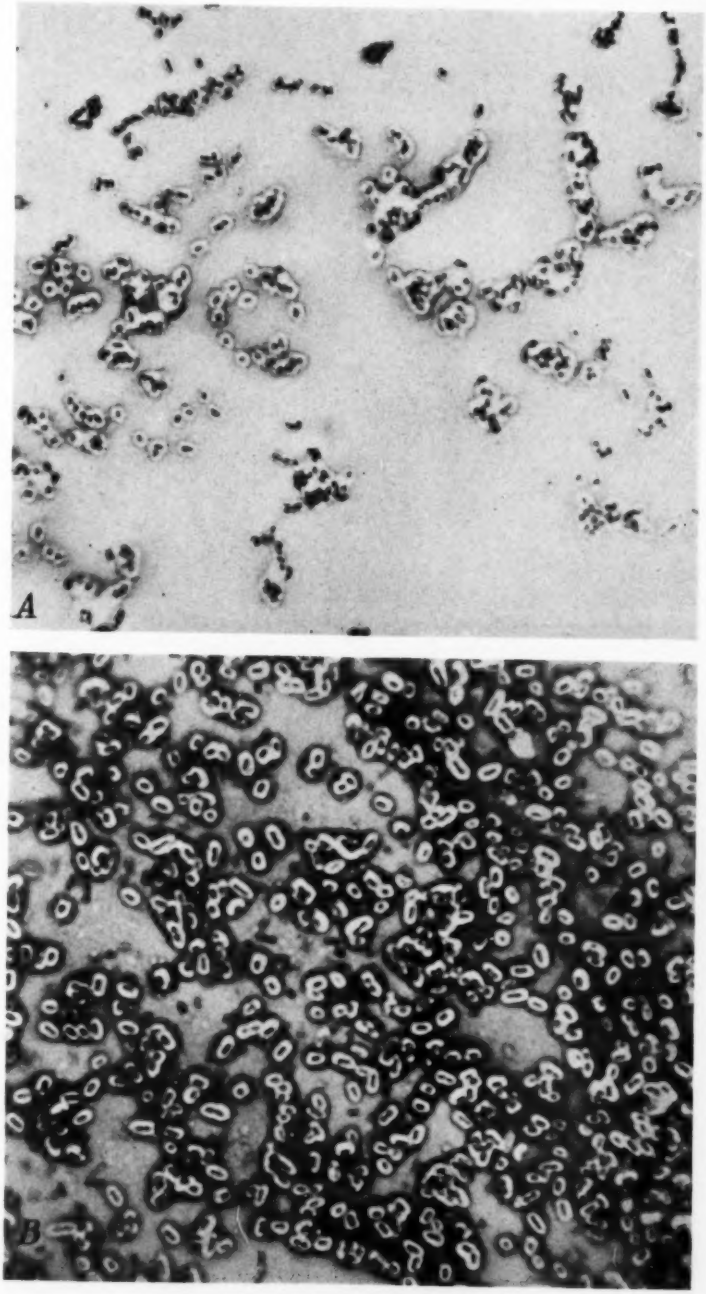
As observed under the microscope, the bacterial bodies within the thread are seen surrounded by a colorless substance taking the form of a halo which appears to be more glistening than the bodies, and which possesses a more or less definite outline. In general, the width of this substance as observed in these unstained, dry threads approximates that of the bodies and surrounds the bodies in much the same fashion as has been described for various encapsulated species of bacteria.

In contrast with these observations, when a drop of nutrient broth or syneresis liquid at the base of an agar slant, representing young, vigorously growing cultues of *Erwinia amylovora* are, without staining, examined in a hanging-drop slide, no such well-defined refractive substance can be seen around the bodies, although there are occasional suggestions of it. In such hanging drops, the bodies of the bacteria are usually much larger than in the dry bacterial threads.

When a droplet of fresh exudate is placed in a drop of sterile water on a flamed slide, the bacteria will shortly cloud the water, indicating a dissolution or separation of the bacteria in the exudate. If this clouded water drop is then smeared over the slide, dried rapidly over a flame, stained for 20 to 30 seconds in a saturated solution of basic fuchsin dissolved in 95-percent alcohol, washed in running water, and examined under the microscope, there are very definite indications of a substance surrounding the bacterial bodies (pl. 1, *A*). If the bacteria are grouped or rather held together, a definite margined colorless substance, quite refractive to stains except at the margin, will be seen as a more or less continuous layer surrounding the group. If the bacteria are well isolated or separated from each other the individual bodies are often to be seen surrounded by the same type of envelope. However, this is not always the case, and the writer is not certain whether in such instances the enveloping layer has been lost somewhere in the process or that the technique does not work the same on all bacteria in a given smear. Since the bacteria representing the outer layers of exudate would be acted upon by the water for a greater length of time than the inner ones, there is of course the possibility that their enveloping layers would be subjected to greater

EXPLANATORY LEGEND FOR PLATE 1

Erwinia amylovora. *A*, Derived from fresh exudate, mounted in a drop of sterile water, dried rapidly over a flame, stained in a saturated solution of basic fuchsin dissolved in 95-percent alcohol, and washed in running water. Note presence of stainless material surrounding the bodies which is taken to be bacterial slime and which suggests capsulelike structures. × 1,725. *B*, Derived from exudate, kept outdoors at a relative humidity of 50 percent for 4 months, indicated the presence of viable and infectious material in inoculation tests, and was stained in the same way as that in *A*. Note presence of stainless material immediately around the bodies followed by an extreme outer layer of staining matter. These resemble encapsulated bacteria. × 1,820.



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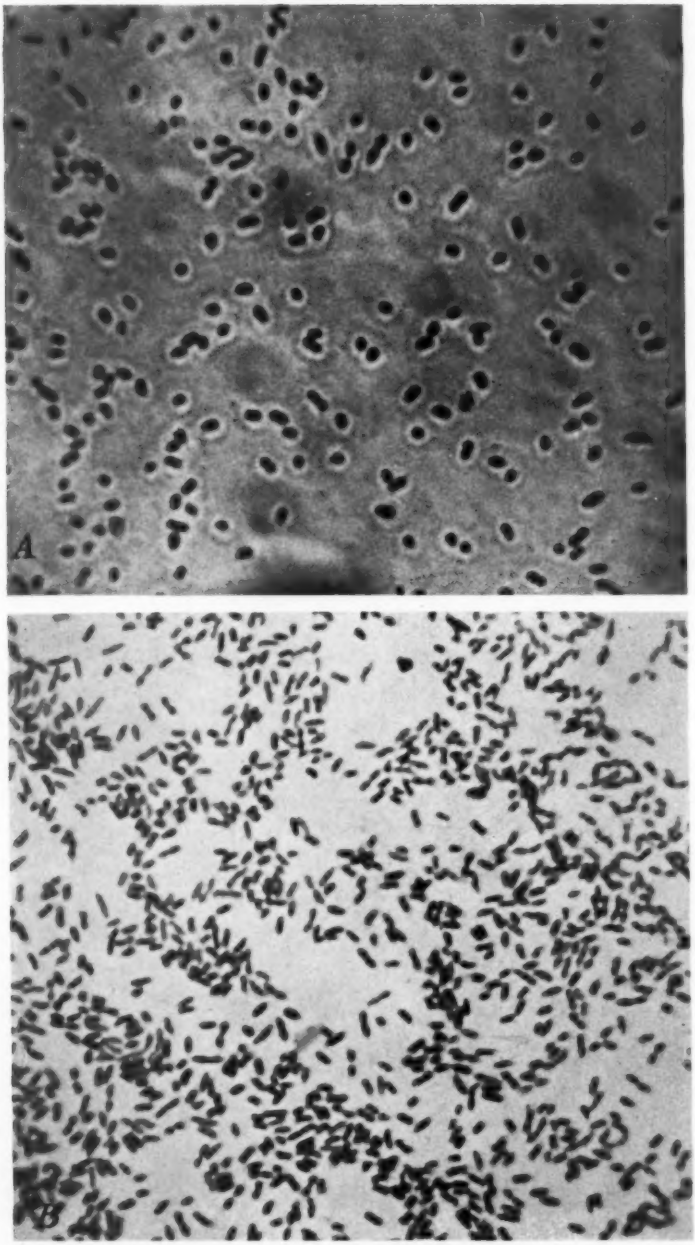

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change. It is well known that slimy envelopes are apt to be dissolved by water. Nevertheless, there is no difficulty whatever in finding many of the bacteria in such preparations with a clear-cut outer layer (pl. 1, *B*). Often the edge of this colorless layer is marked by a substance which is stained by the basic fuchsin, though not as deeply as the main bodies located within the colorless layer. This substance at the outer edge of the nonstaining layer is exceedingly elusive, often not at all observable or present merely as a trace (pl. 2, *A*). When this occurs, there is little or nothing to mark the outer edge of the colorless material unless some substance such as india ink or other extraneous matter is present which possesses an entirely different refractive index or stains readily. In the absence of any stained material at the margin of the stainless substance, the latter is practically unobservable.

CONTRAST IN STAINING REACTIONS

Contrasted with the highly refractive and often sharply delimited stainless material surrounding the bacterial bodies found in bacteria derived from exudate, no such well-defined nonstaining material is to be observed in bacteria derived from nutrient agar or broth cultures (pl. 2, *B*), although not infrequently there occur stainless areas around the bodies which suggest that they are not mere artifacts or light diffraction patterns but reduced or modified layers of comparable material found in exudate. Furthermore, old viable nutrient-broth cultures kept in a refrigerator will often show more or less of a viscidity when touched with a needle or loop, depending partly on the amount of agitation to which the cultures have been subjected. If such cultures are shaken vigorously, the viscidity especially of the upper liquid is readily lost, suggesting a dissolution or separation of the slime from the bodies, part of the slimy matter settling toward the bottom of the cultures. Upon decanting the upper liquid, the precipitate at the bottom still possesses some viscidity, varying apparently with the amount of agitation. Old, undisturbed cultures, in which most of the liquid has evaporated and which still possess viable bacteria, often show a degree of viscidity approaching that of bacterial ooze exuded from host tissues. There is, therefore, little doubt that *Erwinia amylovora* produces slimy matter in such cultures, although the slime is soon separated from most of the bodies, the separation depending upon the amount of water present and the degree of agitation.

In view of the fact that *Erwinia amylovora* has been described by various investigators as having no capsules, what are the enveloping layers, which are readily observable in bacteria derived from exudate—both in living material and in stained preparations?

The presence of these enveloping layers in living material suggests that they are not artifacts. Nevertheless, to make sure of this, fresh sticky exudate was placed on a glass slide in a mixture of india ink and water, the bacterial-ink preparation smeared over the slide with the edge of another slide as in making a blood smear, dried rapidly over a flame, stained with saturated basic fuchsin in 95-percent

EXPLANATORY LEGEND FOR PLATE 2

Erwinia amylovora. *A*, Derived from fresh exudate, mounted in 60-percent alcohol, dried, and otherwise handled like that shown in plate 1, *A*. Note reduced amount or absence of dark-staining matter at the outer margins of the colorless zones surrounding the bodies. Compare with plate 1, *B*. × 2,415. *B*, Derived from a 48-hour-old nutrient-agar-slant culture treated the same as that shown in plates 1 and 2, *A*, with which compare. Note very slight suggestion or none at all of slime or capsule formation. × 2,027.

alcohol, and washed in tap water. Preparations made in this manner almost invariably show a red-staining bacterial body surrounded by a well-defined colorless layer which is sharply delimited by the particles of india ink (pl. 3, *A*). The same structures appear in india-ink preparations without the use of heat or of staining, although the interior bodies being unstained or slightly so are not to be clearly distinguished, for photographic purposes, from the surrounding colorless layers. When nutrient agar cultures are treated with india-ink water mixtures there are also suggestions of colorless enveloping layers or capsules around the bodies but these layers are rarely ever as well defined or as large as in the bacteria derived from fresh exudate or from hard, dry exudate which contains living bacteria. Indeed, many of the bacteria derived from pure cultures in artificial media show no evidence of capsule or slime formation, although the evidence is apparently sufficient to indicate that *Erwinia amylovora* in pure cultures, especially in nutrient broth, produces some slime which is either readily shaken off or partially dissolved from the bodies of many of the bacteria. In this respect this species behaves somewhat differently from those human and animal bacterial pathogens which have been described as being encapsulated within the host and not in artificial cultures. The well-known behavior of *Bacillus anthracis* Koch is a good illustration of this (*6, p. 39*).

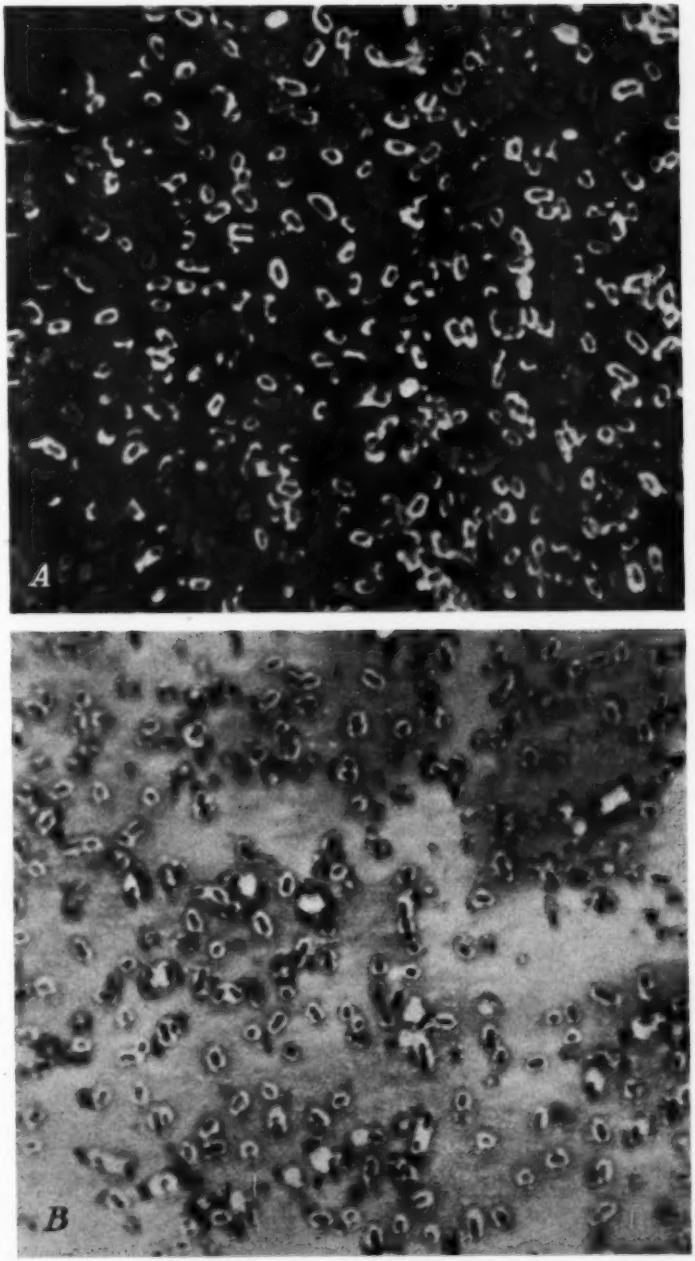
OTHER EVIDENCES OF ENVELOPES IN NATURAL EXUDATE

In addition to the above evidence for the presence of an enveloping layer around fire blight bacteria, especially those derived from exudate, there are two other observations which indicate the same thing. Since artificial cultures usually yield relatively few bacteria with definite indications of such layers, it seems fair to assume that the water contained in such cultures is probably responsible for their removal or partial disintegration. To test this theory, droplets of exudate were placed in sterile distilled water, using 2 droplets for approximately 1 cc, allowed to stand for 1 hour, the resulting cloudy suspensions smeared over glass slides, dried rapidly over a flame, stained with saturated basic fuchsin dissolved in 95-percent alcohol for 30 seconds, and washed with tap water. When such preparations are compared with similar exudate treated with a small drop of water for 2 or 3 minutes prior to the drying of the films, those which have stood 1 hour in water show enveloping layers that are wider but much less refractive and the outer edges are quite indefinite.

Still another line of evidence consists in the observation that bacterial bodies (protoplasts) are not infrequently entirely absent or located much to one side of stainless areas which assume the same general shapes and dimensions of the enveloping layers found in those bacteria with bodies intact and centrally located. There are several highly refractive, stainless areas without bodies or with bodies askew to be seen in plates 1, *A* and 3, *A*, and especially in plate 3, *B*. These

EXPLANATORY LEGEND FOR PLATE 3

Erwinia amylovora. *A*, In fresh, sticky exudate placed in a mixture of india ink and water, smeared over glass slide as in making a blood smear, dried rapidly over a flame, stained with a saturated solution of basic fuchsin dissolved in 95-percent alcohol, and washed with tap water. Note stainless layers around the bodies suggesting capsules or slime formation. × 2,500. *B*, From fresh exuate mounted in a drop of 60-percent alcohol, dried rapidly over a flame, and stained like that shown in plate 1, *A*. Note stained protoplasts centrally located in most instances, surrounded by stainless areas. Note also others in which the protoplasts are considerably to one side or outside of the stainless portions. × 1,725.



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areas apparently are not artifacts since a more or less regular series can be detected, varying from those in which the stained, centrally located bodies are evenly surrounded by the stainless halos, grading to those in which the bodies are decidedly to one side and extending to others in which the dark-staining bodies are entirely outside of the stainless areas. If stainless halos as a whole were mere artifacts consisting of a shrinking of the protoplasts and leaving an outer wall or layer of protoplasm separated from the main body by an unoccupied and hence stainless area, it is obvious that the shrinking occurred prior to the application of the stain; otherwise they would not appear. But, as there are numerous bodies appearing wholly outside of the stainless areas or to one side within the stainless portion, it seems clear that in these instances the bodies were probably dislocated or moved out of their natural position after the application of the stain. This is easily accomplished if the washing which follows the stain is too violent or prolonged, or if the cover glass is moved in the process of mounting, and various preparations have been obtained in which almost all the bodies were washed away or dislocated, leaving at times mere outlines of the enveloping layers.

If the stained protoplasts, centrally located or otherwise, represented shrunken bodies, they should appear more or less irregular in outline with disorganized parts evident, and staining irregularly. No such protoplasts have appeared in any of the preparations made for determining the presence or absence of enveloping layers by the method described above. As may be seen in any or all of the figures, protoplasts in sharp focus show definite and regular outlines and are stained homogeneously or with the well known bipolar staining reactions typical of normal bacteria. While the stainless zones around the protoplasts show considerable variation in size and shape, the protoplasts themselves are almost always regular. Furthermore, the size of these protoplasts plus the enveloping layers is considerably larger than those obtained for bacteria derived from the same exudate but stained with ordinary methods, such as Ziehl's carbol fuchsin, which do not reveal the outer layers. Often the bacteria which show these envelopes are fully twice as wide and as long as those which show no envelopes.

NATURE OF ENVELOPES

How to designate these outer slimy layers of *Erwinia amylovora* is a difficult question. Benecke (*3*) distinguishes two different types, the true encapsulated bacteria which produce gelatinous layers (Gallertbildung), and those which produce slimy layers (Schleimbildungen). The latter are distinguished by the ability of these layers to swell and to be soluble or deliquescent. However, he indicates that there are no clear distinctions between capsules and slimy layers (*3, p. 94*) and since he proposed no distinct term for the latter, the writer is inclined to call the slimy envelopes of *E. amylovora* "capsules" for want of a better term. Certainly, as observed in dry living threads, the bacterial protoplasts show these enveloping layers between them as structures just as fixed as in the recognized encapsulated bacteria. However this may be, it would appear far more important to recognize the fact that fire blight bacteria when derived from natural hosts, in the form of exudate, are encased in slime than to insist that these slimy secretions be called capsules.

The chemical nature of these slimy layers has not been fully investigated. Since the outer edges often take the basic fuchsin stain when the main part does not, there is a suggestion that the outer edge is in part proteinaceous. Likewise, since the greatest part takes no protein stain, it is probably mainly carbohydrate in composition. This appears to be in keeping with the findings of other investigators who have studied the composition of slimy bacterial envelopes. Ward (*20, p. 7*) in reviewing these studies calls attention to the following: In the Friedländer bacillus (*Klebsiella pneumoniae* Trevisan), Toenniessen has shown that the slimy envelope or capsule is made up of galactan and contains no protein. This was confirmed by Kramár who, in addition found that the capsule of *Bacillus anthracis* consisted of glycoprotein while that of *B. radicicola* Beijerinek was made up of a dextran. Likewise, Avery, Heidelberger, and Goebel found the capsular substance of pneumococcus type II to be a nitrogen-free polysaccharide and they found a similar carbohydrate in a strain of the Friedländer bacillus. Finally, in studying certain encapsulated strains of *B. coli* Migula, Smith found the capsule material to consist of 80 percent of hexose and a small amount of glycuronic acid. In short, all investigators here mentioned have found that slimy bacterial envelopes are composed either wholly of carboydrates or carbohydrates combined with proteins.

The conclusion is that the bodies of fire blight bacteria in the form of exudate derived from natural hosts are enveloped by slimy capsules and that this slime consists mostly of nonproteinaceous material. On the other hand, fire blight bacteria maintained as pure cultures in nutrient agar or broth rarely show such slime around the bodies. This is probably the reason why pure cultures show little or no viscidity when fresh exudate reveals it in striking form.

It is also concluded that the presence or absence of the slimy layers around the bodies probably exercises considerable influence on the longevity of the bacteria and on their ability to live under diverse conditions of temperature and relative humidity. At any rate, as shown in table 1, bacteria without slimy envelopes, those derived from nutrient agar, are very short-lived under the same conditions of temperature, humidity, and light that are conducive to exceptional longevity of bacteria that are encased in slimy envelopes derived from exudate.

DISCUSSION

The results obtained in subjecting bacterial exudate of *Erwinia amylovora* to a relatively wide range of temperatures and to various relative humidities indicate that neither of these factors taken by itself, within the ranges utilized, exercise an appreciable deleterious effect on the parasite, at least not for relatively long periods of time. To find a supposedly nonspore-forming species of bacteria capable of living at a temperature of 104° F. for at least 400 days, is in itself rather surprising since micro-organisms as a whole have been found in numerous investigations to be adversely affected by such high temperatures and rather resistant to temperatures much lower than the lowest utilized in the studies here reported.

These results are seemingly in disagreement with previous studies on thermal death-point determinations of *Erwinia amylovora* (*1, 14*) in which it was found that this parasite is quite sensitive to high temperatures, perishing in a few minutes when subjected to 43.7°,

45°–50°, 45°–46°, and 45.1°–48.3° C. for some isolates and 48.3°–49.5° for others, as found by different investigators. Actually, however, the writer is convinced that the disagreement may readily be explained by the difference in materials and methods rather than by any discrepancy in results. The five previous investigators all relied on a standardized technique based upon the use of such an artificial medium as peptone-beef broth or synthetic media. Clearly there must be considerable difference between the physiology and probably the morphology of a living thing growing in beef broth compared to the same organism which had made its growth within living host tissues and had been extruded from such tissues. The studies here reported on slime formation certainly suggest such differences.

The fact that at a relatively constant temperature of 40° C. (actually at least 4° higher at times), and at low relative humidities, the bacteria remain viable for at least 400 days suggests that when they are studied in the form of natural exudate the thermal death point will be found to be considerably higher. In any case the studies here reported not only indicate that *Erwinia amylovora* can withstand high temperatures for relatively long periods, but also suggest that the standard methods utilized for determining thermal relationships of bacteria in general are of doubtful value so far as indicating their reaction toward heat when they are grown under natural conditions.

While neither the temperatures nor relative humidities alone exercised a deleterious effect on *Erwinia amylovora* over relatively long periods, the data show as previously indicated that there is a very appreciable effect at higher temperatures when the relative humidities approach 45 percent. The interaction between moderate or high temperatures and high relative humidities apparently bring about a condition in which the bacteria are short-lived.

What is the possible significance of such behavior from the standpoint of available inoculum carried from one season to another? It is evident from these studies that too little attention has been given in the past to the possibility of this parasite being carried over in the form of exudate from one year to another. Almost all investigations on fire blight rest on the assumption that the inoculum is carried within host tissues blighted the year previous, in so-called hold-over blight. Why the bacteria should be considered as more likely to survive within host tissues than on the outside is not very clear. Aside from the influence of direct sunlight on exposed bacteria, the temperature of intercellular spaces, the main channels of bacterial invasion of host tissues, are probably proportional to those of the air. In strong light Curtis (*4*) found the internal temperature of apple leaves to be actually higher than the air temperature, while in diffuse light it was lower. In both instances the internal temperature was found to be directly proportional to that of the air. Similarly, Miller and Saunders (*9*) found the curves of surface temperature of leaves of various plants to follow those of air temperature pretty closely through a 24-hour period. With some plants the surface leaf temperatures are a little higher and in others somewhat lower than those of the air. So far as relative humidities of air compared with that of intercellular spaces are concerned, the writer has found no

definite data, although Shaw's studies (*17*) suggest that in succulent twigs of apple and pear the relative humidities are often much higher than that of the air. If this is true, then in accordance with the findings here presented the bacteria may be expected to be shorter-lived within the tissues than in extruded ooze since combinations of high relative humidities and moderate or high temperatures would occur more frequently within tissues than in the air of at least part of the fruit-growing areas found in this country. This, however, remains to be determined.

Considering the influence of humidity and temperature, the longevity of the bacteria as extruded ooze may be expected to be different in different sections of the country, depending at least in part on prevailing temperatures and relative humidities. Likewise, the longevity within tissues compared to that in exudate may be different.

The writer's studies of the longevity of *Erwinia amylovora* other than within host tissues have received encouragement in several interesting and promising lines of investigation, including the recent report of Ark and Thomas (*2*) on the persistence of this parasite in both fruit-flies and houseflies from pupa to adult, and also on the eggs of the latter. As such studies progress and include bacteria produced under natural conditions instead of artificial cultures, one may confidently expect to find these bacteria living for relatively long periods within a rather wide range of climatic conditions, in accordance with the studies here presented.

In addition to beehives it is obvious that any other nidus in and around orchards, where exudate may be carried by other insects or by rain and wind and where it may be shielded from direct rays of the sun, requires investigation before it can be certain that the bacteria are not living from season to season outside of host tissues.

SUMMARY

A study of the combined influence of temperature and relative humidity on fire blight bacteria in the form of natural exudate and as pure cultures revealed the following:

At a comparatively low, controlled temperature (16° C.) the bacteria as exudate remained viable and infectious at the four different relative humidities utilized (approximately 0, 9.5, 21.0, and 45 percent), throughout the entire period of testing, over a year.

At moderate and high, controlled temperatures (25°, 30°, 35°, and 40° C.) the bacteria in exudate also remained viable for long periods when the relative humidity was low. At 45 percent relative humidity they were dead when the first tests were made. It seems, therefore, that a combination of high temperature and moderate or high humidity is conductive to short life in this species of bacteria, while equally high temperatures with low humidities make for long life.

At uncontrolled or fluctuating temperatures, obtained by placing the exudate outdoors or on the laboratory shelf, the bacteria remained viable outdoors at 50 percent relative humidity for the entire period of testing, over 9 months, and at approximately zero relative humidity for almost 1 year, while at 75 and 90 percent relative humidities they died rapidly outdoors. At fluctuating laboratory temperatures the bacteria remained viable at approximately zero relative humidity for as long as the test was conducted, 610 days.

Contrasted with the long life of the bacteria in the form of exudate under fluctuating laboratory conditions, the bacteria obtained from artificial cultures are short-lived under the same conditions.

The longevity of fire blight bacteria within blighted host tissues has been found to be approximately equal to that of the bacteria in the form of exudate when the humidity is low.

Fire blight bacteria in the form of minute droplets of exudate were found to be viable and infectious at the end of 22 days when immersed in honey contained within comb kept outdoors in United States Weather Bureau-type housing. They were not viable at the end of 92 days under these conditions. As present within bits of diseased host tissues, also immersed in finished honey and otherwise under the same conditions as the exudate, the bacteria were found to be viable at the end of 121 days.

A morphological study of the bacteria derived from exudate indicates that they are enveloped in slimy capsules which are mainly non-proteinaceous. Similar studies of the bacteria derived from pure cultures also indicate that on artificial media such slimy layers are either dissolved or greatly reduced.

It is concluded that the presence or absence of slimy envelopes probably exercises considerable influence on the longevity of the bacteria under diverse conditions of temperature, humidity, and light; and that the bacteria encased in slimy layers, as they are in natural exudate, offer a fertile field of investigation relative to the epidemiology of fire blight, in the light of the studies here reported.

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FACTORS INFLUENCING THE DISCOVERY OF FOREST FIRES BY LOOKOUT OBSERVERS[1]

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INTRODUCTION

In forest-fire suppression activities there are four generally recognized elapsed-time steps between the start of a fire and the time suppression forces actually begin working on it. These are (1) discovery time, (2) report time, (3) get-away time of the initial attacking force, and (4) travel time of attacking force. To attain the promptness of attack desirable—termed "hour-control requirements"—in any given forest cover type, it is necessary to keep the sum of these four elapsed-time steps less than the total allotted in the hour-control set-up.[3]

The discovery time of fires, under the type of detection system employed throughout the national forests of California,[4] is subject to considerable variation, attributable to the varying physical conditions under which fires occur. This variation, unlike that in the other three elapsed-time steps, cannot be controlled by the protection organization. It is, however, desirable to measure the extent of the variations which occur and to associate them with their controlling physical factors in order that significant variations in discovery time may be estimated from the measurement of physical conditions under which fires occur.

The purpose of this paper is to present some measurements that have been made of the influence of certain factors on discovery time in the ponderosa pine (*Pinus ponderosa* Dougl.) type of northern California. While the quantitative experimental results obtained may not have direct application to other cover types or other forest regions, the trends established by the study may be of some value to all engaged in fire-control activities.

METHOD OF STUDY

In this study 200 actual test fires were set and allowed to burn from 10 to 30 minutes, depending upon their rate of spread, on the McCloud Flats of the Shasta National Forest during one fire season between July 1 and October 15.

Observations of the fires and quantitative measurements of the variables were obtained by two groups of observers. One, the ground crew, started and suppressed the fires and recorded weather and fuel data and the behavior of the fires. The perimeter of each fire was

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[2] Maintained in cooperation with the University of California, at Berkeley, Calif.

[3] SHOW, S. B., and KOTOK, E. I. THE DETERMINATION OF HOUR CONTROL FOR ADEQUATE FIRE PROTECTION IN THE MAJOR COVER TYPES OF THE CALIFORNIA PINE REGION. U. S. Dept. Agr. Tech. Bull. 209, 47 pp., illus. 1930.

[4] BROWN, A. A. IMPROVING FOREST FIRE DETECTION IN CALIFORNIA. Jour. Forestry 33: 923-931. 1935.

plotted to scale at 2-minute intervals during the life of the fire. The second group consisted of a special observer on Black Fox lookout in the center of the area in which the fires were set and cooperating lookout observers on four other lookout points from each of which most of the area was directly visible. Each test fire set thus represented from two to five separate and independent discoveries. The special observer on Black Fox recorded the position of the sun at the start of each fire, a description of the background against which the smoke appeared, visibility conditions as indicated by visibility-meter ratings of natural objects in the landscape,[5] and discovery time. The cooperating lookouts recorded a description of backgrounds, general estimations of visibility conditions, and discovery time. Radio communication was maintained between the ground crew and Black Fox, and all lookout observers were notified in advance of the location and time of start of each fire. No attempt was made in this study to check the personal efficiency of the different observers.

The fires were confined mainly to pure ponderosa pine needles. This type was selected as being the most uniform in fuel character and the one in which the fires could be most easily suppressed. Analysis of the data pertains only to the pure pine type.

EFFECT OF FACTORS ON DISCOVERY TIME

Collection of field data was limited to fires that spread immediately upon ignition. The time in which an incipient fire in the form of a smoldering campfire or rotten log will actually start to spread and produce smoke is governed more by chance than by any measurable physical factors. Lookouts can normally be expected to discover only such fires as are spreading and have produced a definite column of smoke.

The important physical factors which influence the time from the start of spread of fires to their possible detection by lookouts are: (1) Atmospheric obscurity, (2) background against which the smoke is seen, (3) distance of the lookout from the fire, (4) relative position of the sun with respect to the observer's line of sight, and (5) relative behavior of the fire governing the rate at which smoke is produced and rises—this last being dependent upon the combined influences of wind velocity, moisture content, and nature and distribution of the fuel. The correlation of fire behavior with these governing factors is being undertaken as a separate project and has not been treated in the study of discovery time. In the collection of the field data, quantitative measurements were made of each of these factors for every discovery recorded, with the exception of background. This factor was described by its composition and appearance to each observer. The effect of topographic shadows upon discovery time was not considered, since they play a very minor role in the detection problem in northern California.

ATMOSPHERIC OBSCURITY

The degree of atmospheric obscurity was measured in terms of the clearness with which objects in the landscape could be seen. Because of suppression of many of the fires before they could be discovered by all the lookouts under poor atmospheric visibility conditions, data on

[5] BENNETT, M. G. A VISIBILITY METER. Jour. Sci. Instruments 8: 123–126, illus. 1931.

the influence of atmospheric obscurity are incomplete. Analysis of all the discoveries recorded, however, shows little quantitative relationship between the visibility of natural objects in the landscape and discovery time. This indicates that the concentration of particles in the atmosphere has a different effect upon the visibility of a smoke column from that upon the visibility of the landscape. Further work is required to find a more satisfactory method of measuring the degree of atmospheric obscurity as an influence on fire detection.

In order to simplify the analysis of the remaining factors, the range of atmospheric visibility encountered was divided into three broad classes based on visibility-meter ratings, and only those discoveries were used that fell within the upper class, representing excellent to moderate atmospheric transparency. This group constitutes approximately 86 percent of the observations made.

BACKGROUND

Green ponderosa pine timber, brush fields, mixtures of brush and timber in various proportions, and open flats covered with dry grass made up the backgrounds against which the test-fire smokes were observed. These were segregated into two classes to which differences in discovery time could be definitely attributed. The first and larger group includes the dark-colored backgrounds of brush, timber, and mixtures of the two. The data show no differences in discovery time caused by differences in background within this group.

The light-colored backgrounds of dry grass comprise only a very small portion of the experimental data and are insufficient in number to permit of detailed analysis. A summary of the data reveals quite definitely, however, that, under the same weather and fuel conditions, discovery time of smokes observed against backgrounds of dry grass is longer and much more erratic than that against the darker backgrounds of brush and timber.

A brief consideration of the factors controlling the intrinsic visibility of objects explains this. An object is visible to an observer because of contrasts in either brightness or color between the object and its background, or because of a combination of the two contrasts.[6] When either type of contrast is of a low degree, the other must be of a much higher degree, to cause the object to be visible. Smokes observed against brush and timber backgrounds are always brighter than their backgrounds and normally present a strong contrast in both brightness and color. Even when one type of contrast is low, the other is usually sufficiently strong to make the smoke visible. Smokes observed against the sky or dry-grass backgrounds, on the other hand, may be either somewhat brighter or less bright than their backgrounds or of the same brightness as their backgrounds, resulting in a low degree of contrast. This, combined with low color contrasts between smokes and light-colored backgrounds, causes such smokes to have a relatively low degree of visibility. Under these conditions such a fire, considerably prolonged, may not be discovered until the smoke column becomes large enough to appear against an adjacent darker background or to become visible through its increased size and

[6] NUTTING, P. G. THE VISIBILITY OF RADIATION. A RECALCULATION OF KÖNIG'S DATA. U. S. Bur. Standards Bull. 7: 235–238. 1911.
——— REPORT OF STANDARDS COMMITTEE ON VISUAL SENSITOMETRY. Jour. Optical Soc. Amer. 4: 55-79, illus. 1920.

changing brightness with increase in volume and density. Analysis of the remaining factors is confined to smokes showing against dark backgrounds.

DISTANCE

It is impossible to isolate completely the factor of distance between fire and observer. In a perfectly transparent atmosphere, the only effect of distance is to control the visual angle subtended by the smoke column at the eye of the observer. Within the limits of the experimental data, this effect is negligible when expressed in minutes of discovery time. Since the atmosphere is never completely transparent, however, the degree of transparency has an important influence on the apparent effect of distance upon discovery time.

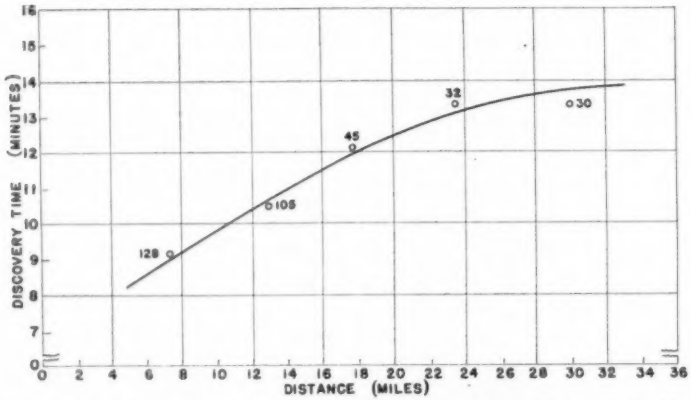

FIGURE 1.—Effect of distance upon the discovery time of pine fires under favorable atmospheric visibility. The figures on the curve indicate the number of observations taken.

The curve in figure 1, showing the increase in discovery time attributed to increases in distance, represents observations made under average good visibility rather than optimum conditions and therefore includes some effect of atmospheric obscurity. Within the limits of 15 miles, which is usually accepted as the maximum effective radius of vision of lookout observers, average discovery time may be considered as a straight-line function of distance under normal conditions. Distances greater than 20 miles, on the other hand, have a decreasing effect on discovery time because fires allowed to burn long enough to become visible at these distances normally begin producing smoke at a greatly accelerated rate. After this acceleration has begun, a large column of smoke is formed which becomes visible at relatively long distances within a very short time.

ANGLE BETWEEN SUN AND OBSERVER

Prior to analysis of the field data, a laboratory experiment was performed [7] to determine the effect of direction of illumination upon the visibility of a smoke column under controlled laboratory condi-

[7] BUCK, C. C., and FONS, W. L. THE EFFECT OF DIRECTION OF ILLUMINATION UPON THE VISIBILITY OF A SMOKE COLUMN. Jour. Agr. Research 51: 907-918, illus. 1935.

tions. From this experiment several conclusions were drawn, the most important of which was that the position of the light source (sun) with respect to the observer's line of sight, together with the concentration of particles in the smoke column, has a very definite effect upon the visibility of the smoke column. The results of this study materially aided in the analysis of this phase of the field data.

Following the trends indicated in the laboratory experiment, the effect of relative position of the sun upon discovery time was determined, as shown in figure 2.

This effect may be expected to vary somewhat with the degree of atmospheric obscurity. This in turn varies with position of the sun in the same manner as does the brightness of a smoke column, since the degree of variation in either is caused by the concentration of particles of suspended matter. The net variation in the visibility of the smoke column and its corresponding discovery time caused by variations in relative position of the sun is, therefore, determined by the relative concentrations of particles in the smoke column and in the atmosphere. Even under very adverse visibility conditions, however, in every observation made in the field with the sun behind the observer the discovery time of the fire was longer by at least a small margin than that of similar fires observed facing the sun.

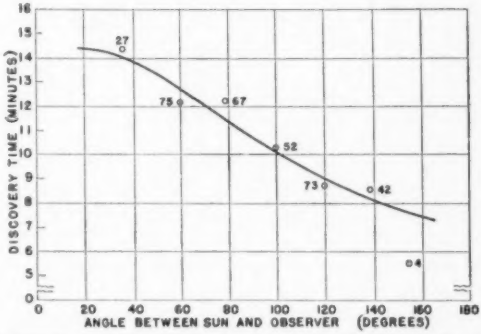

FIGURE 2.—Effect of relative position of the sun and observer's line of sight upon discovery time of pine fires under favorable atmospheric visibility. The figures on the curve indicate the number of observations taken. This is the angle with the smoke at the vertex and is the supplement of the angle measured at the observer between the sun and the smoke.

RATE OF SPREAD

Rate of spread is the principal element of fire behavior which influences the time required from the start of a fire for its smoke column to build up to the volume and density necessary to become visible to a lookout observer. Despite certain disadvantages, the unit of measure of this factor in the analysis was taken to be the average rate of spread for the first 8 minutes, since all fires were allowed to burn for at least this period. As a measure of rate of spread, average diameter, total perimeter, and area in square feet were all tried, but the last proved the most satisfactory in relation to discovery time. No usable relationship was found to exist between the size of a fire at any given moment and the size and density of its smoke column.

Figure 3 shows the average effect of the rate of spread of pine fires upon their discovery time under favorable atmospheric visibility. It will be noticed that discovery time decreases only slowly after rates of spread of approximately 40 square feet per minute are exceeded. This is due in part to the increased wind velocity responsible for the increased rate of spread, which undoubtedly has a marked influence

in keeping the smoke from rising above the timber crowns where it can be seen. Further, a rather definite minimum discovery time is determined by the failure of a fire to produce smoke for the first 1 or 2 minutes.

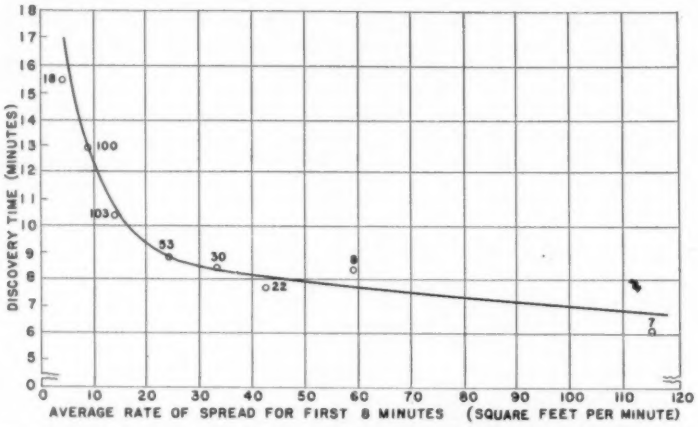

FIGURE 3.—Effect of average rate of spread upon the discovery time of pine fires under favorable atmospheric visibility. The figures on the curve indicate the number of observations taken.

COMBINED EFFECT OF RATE OF SPREAD, DISTANCE, AND POSITION OF THE SUN ON DISCOVERY TIME

To show graphically the combined influence of distance, position of the sun, and rate of spread upon discovery time, it is necessary either to make use of three-dimensional figures or to represent the influences of two of them with a single term. Analysis of the individual effects

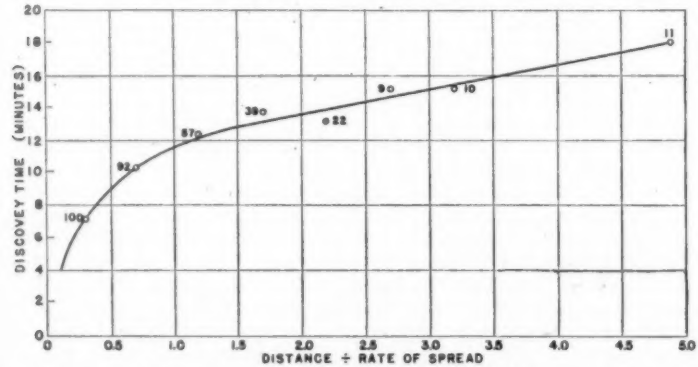

FIGURE 4.—Discovery time expressed as a function of the ratio between distance in miles and average rate of spread in square feet per minute for the first 8 minutes. The figures on the curve indicate the number of observations taken.

revealed that the latter method, involving the expression of discovery time as a function of the ratio between distance and rate of spread, could be used satisfactorily within the limits of the experimental data. This relationship is shown in figure 4 and indicates that under similar

conditions of position of the sun and atmospheric visibility, the discovery time of a fire 10 miles distant burning at an average rate of 25 square feet per minute for the first 8 minutes will be the same as that of a fire 20 miles distant burning at an average rate of 50 square feet per minute.

In figure 5, average rate of spread for the first 8 minutes and distance are represented on logarithmic scales in *A*. The ratio of distance to rate of spread is represented on a logarithmic scale by the diagonal lines in *A* which become the abscissae in *B*. Discovery time for different angles between sun and observer is plotted as the

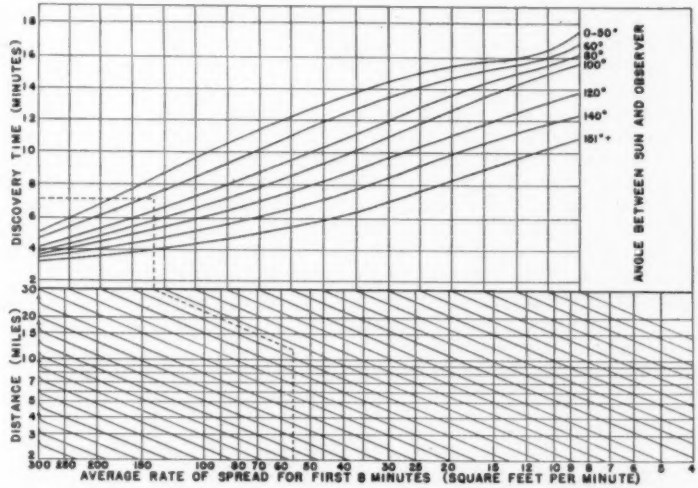

FIGURE 5.—Combined effects of average rate of spread, distance, and angle between sun and observer upon the discovery time of pine fires observed against brush and timber backgrounds under favorable atmospheric visibility. Example (shown by dotted line): Rate of spread 55 square feet per minute, distance 12 miles, angle between sun and observer 65°; discovery time 7.1 minutes.

ordinate in *B*. A total of 340 discoveries form the basis of this set of curves. Discovery time may be estimated from figure 5 in the manner illustrated by the broken line in the diagram.

Example: Rate of spread, 55 square feet per minute; distance, 12 miles; angle between sun and observer, 65°. Start at the value 55 on bottom scale and read vertically to distance 12 on lower left-hand scale; from this point follow upward to the left, parallel to the diagonal lines, to the heavy horizontal line, then vertically to the value 65° obtained by interpolating between the curves for 60° and 80°; discovery time is read on the upper left-hand scale opposite this point, 7.1 minutes.

A comparison of actual discovery times with estimated values from figure 5 indicates a relatively high degree of accuracy for observations during the middle of the day. Observations made at 7, 8, and 9 a. m. are more erratic. The heat from early morning fires is usually insufficient to puncture the inversion layer which characteristically persists in the experimental area from 15 to 30 feet above the ground at that time of day. The rising column of thin smoke from these early morning fires, upon striking the inversion layer, spreads out in

a horizontal plane and is soon dissipated. Discovery times of these fires may exceed the estimated values by as much as 10 minutes. Deviations of midday observations from the estimated values may nearly all be attributed to atmospheric obscurity. The experimental observations cover a range of distances from 3 to 33 miles and at the upper limits of this range even a slight haziness in the atmosphere undoubtedly has a detrimental effect upon discovery time.

SUMMARY

Better planning of protection organization developments and activities and better utilization of available man power are promoted by providing a more certain definition of elapsed-time allowances between start of fires and discovery than can be obtained from individual fire records. Solution of the discovery-time problem can contribute materially to this end. As a first step in this direction, the three discovery-time factors—distance, rate of spread, and position of the sun—were measured and their interrelation with respect to discovery time determined. The importance of these measurements, as shown in the accompanying charts, is that they form a basis for future work and at the same time may be used as trends indicating relative variations in discovery time which may be of value in present fire-control planning work. Their value will be enhanced materially as quantitative measurements are made of the effects of each of the remaining factors.

MECHANOGRAPHIC METHOD OF RECORDING INSECT CARDIAC ACTIVITY, WITH REFERENCE TO EFFECT OF NICOTINE ON ISOLATED HEART PREPARATIONS OF PERIPLANETA AMERICANA[1]

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INTRODUCTION

It is well known that many substances and experimental treatments may alter both the rate and the amplitude of heartbeat. This has been observed in a recent study of the effects of nicotine upon the contraction rate of isolated insect heart preparations (*6*)[3], in which a previously described method was used (*7*). That method yielded rate measurements only, and it was desirable to have a method that would measure both contraction rate and amplitude.

The present paper contains a preliminary description of a method by which rate and amplitude of the isolated insect heart preparation can be recorded photographically and measured. The results of the method are illustrated by tracings (mechanocardiograms) obtained from various segments of entire untreated and nicotinized isolated heart preparations of the American cockroach, *Periplaneta americana* (L.), and from completely isolated single segments.

METHOD

The mechanocardiograph method consists in transmitting the movement of the insect heart by means of a hair to a glass lever with an opaque long arm that moves in the path of a beam of light. The shadow of the moving arm is magnified and thrown across the slit of a camera containing a strip or roll of bromide paper. As the shadow moves, a tracing (mechanocardiogram) of the heartbeat is recorded upon the light-sensitive paper.

With the exception of the electrocardiographic camera, the set-up necessary for this method can be made from apparatus to be found in the ordinary physiological laboratory. The apparatus used includes the following parts:

The heart lever (fig. 1) consists of a capillary glass tube (*l*) of approximately 0.2 to 0.3 mm diameter drawn out to a smaller capillary tip (*ct*) at one end, so that the shadow of the capillary tip has a diameter of about 1.5 mm when magnified by a compound microscope with 16-mm objective and 10× ocular (tube length of 17.5 cm) and projected upon a screen 14 cm distant from the ocular. The lever is mounted with wax (*w*) upon and at right angles to a short but wider capillary

[1] Received for publication September 18, 1937; issued April 1937.

[2] The author is indebted to A. G. Galloway for his aid in connection with the modification of the electrocardiographic camera used in this work and to Saburo Katsura, of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, for the translation of Uramoto's article (*5*).

[3] Reference is made by number (italic) to Literature Cited, p. 275.

glass tube (*t*) of about 0.8 to 0.9 mm diameter, through which is extended an insect pin (*p*) of greater length and smaller diameter. The smaller end (*ct*) of the capillary lever is part of the long arm (*la*), and the larger end forms the short arm (*sa*) of the heart lever. If the lever is to be used horizontally and its shadow thrown across the vertical slit of an improvised kymographic camera (described below), the end of a straight human hair (*h*) is attached by means of wax to the short arm near the fulcrum, but if the lever is to be used vertically and its shadow thrown across the horizontal slit of an electrocardiographic camera, the end of the hair is attached (at *a*) to the wax (*w*) used to hold the lever to the supporting tube, so that the hair and lever are on opposite sides of the supporting tube (*t*). In either case a very small piece of lens paper (*lp*) is attached by means of a small bulb of wax (*b*) to the other end of the hair. The extremities of the insect pin (*p*, *p*) rest upon a support (*s*); a pair of curved forceps, held in a flat-jawed clamp, with the concavities of the jaws uppermost, which in turn is clamped to a heavy laboratory stand, can be used to support the insect pin. Special care is required to prevent vibrations of the table or building from being transmitted to the lever. The long arm of the lever should be rendered opaque, and in these experiments this was done by allowing Wright's blood stain to dry upon its surface. The exact balance of the lever necessary for the proper recording of heart movement can be obtained by trial.

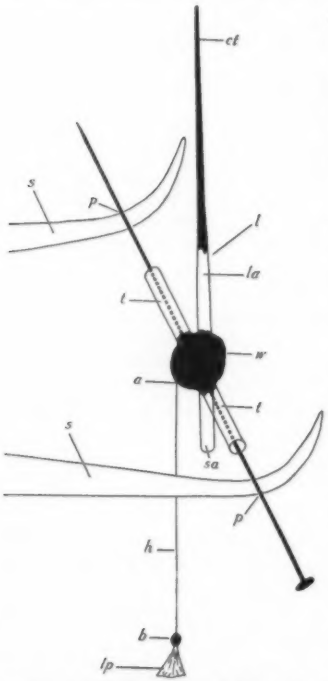

FIGURE 1.—The mechanocardiographic lever, showing its position when in use and held by forceps above the isolated heart preparation (not indicated): *a*, Point of attachment of hair to wax; *b*, wax, holding lens paper to hair; *ct*, opaque tip of heart lever; *h*, hair; *l*, heart lever; *la*, long arm of heart lever; *lp*, lens paper, used to make contact with heart; *p*, insect pin, at point of support; *s*, prong of lever support (forceps); *sa*, short arm of heart lever; *t*, supporting capillary tube of heart lever; *w*, wax, holding heart lever and hair to supporting capillary tube.

The relation of the heart lever to the other apparatus used in the setup is shown in figure 2. The beam of light (*b*) should be intense but with the heat removed by a water cell. The light is concentrated in the region of the tip (*ct*) of the long arm of the lever by a lens (*c*); the lower lens of a 10× ocular can be used for this. The source of light may be an improvised lamp containing a sufficiently strong light bulb, a lens, and a cooling water cell, or a manufactured research microscope lamp containing these parts.

The light, concentrated at the lever tip (*ct*), is passed into the 16-mm objective (*ob*) of a standard microscope tube removed from

its rack-and-pinion support, and clamped in a horizontal position to a heavy iron support; it passes out of the tube through a 10× ocular to the slit of the camera (*s*), which is placed at the desired distance from the ocular and across which the vertical lever shadow (*sl*) falls. These parts are so arranged that the beam of light is centered with respect to the optical system employed.

The camera can be improvised by placing a kymograph (a single-drum student kymograph has been used in some of these experiments) inside a box container that permits no light to enter when completely closed. A vertical slit, covered by a movable lip when not in use, is located in the wall of the container at such position that, when the lever's shadow is thrown across it, the section of the shadow that passes through the slit will fall upon a strip of bromide paper fastened about the kymograph drum and held only a few millimeters back of the slit. The kymograph can be started and stopped by means of a wire that runs from the outside through a small hole in the wall of the container to the starting mechanism. When the lever shadow is focused properly upon the closed slit, the kymograph is started, the slit opened, and the mechanocardiogram obtained; the slit is then closed immediately and the kymograph stopped. An improvised camera of this type is not satisfactory, for several reasons: The strip of bromide paper available for one continuous record is too short; focusing of the lever shadow is partly a matter of guesswork; and the entire camera must be transported to the dark room for loading and unloading. Although mechanocardiograms can be obtained with this improvised apparatus, it is much better to use a standard electrocardiographic camera that has been so modified as to run at required speeds. Most of the records obtained in the experiments reported here were made with a Hindle electrocardiographic camera (fig. 2, *e*) modified to yield paper speeds approximately of from 5 to 65 mm per second. In general, for work with insect hearts it is desirable to have available paper speeds of from 0.5 to 100 or more millimeters per second. Time markings can be obtained by using a watch time marker (*tm*) with a piece of black or opaque paper (*p*) attached to the tip of its recording arm (*a*) and arranged in such manner that the beam of light is interrupted at the required intervals (1 second, 5 seconds, etc.). A synchronous rotator with sets of rotating arms, such as is used with electrocardiographs, is preferable.

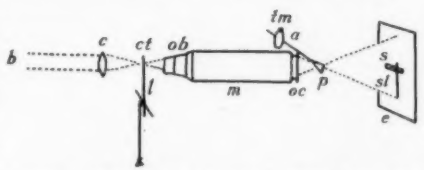

FIGURE 2.—Set-up of mechanocardiographic apparatus: *a*, Recording arm of time marker; *b*, beam of light; *c*, lens, used to condense light at heart-lever tip; *ct*, opaque tip of heart lever; *e*, slit-containing face of electrocardiographic camera; *l*, heart lever; *m*, microscope tube, with attached objective and ocular; *ob*, low-power objective on microscope tube; *oc*, 10× ocular in microscope tube; *p*, opaque paper on time-marker arm; *s*, slit in electrocardiographic camera; *sl*, shadow of opaque tip of heart lever; *tm*, watch-type of time marker.

The isolated heart preparation is dissected out in the manner described previously (7) and is pinned, ventral surface up, to the bottom of a suitable depression cut in a block of wax. The lever is attached to the heart by placing the lens paper (fig. 1, *lp*) on the dorsal dia-

phragm in the midregion just above the ventral surface of the cardiac wall. The tissues are bathed with physiological saline solution (1.25/11 Lévy's stock solution (*4*) has been used for *Periplaneta americana*) applied with a medicine dropper. In these preliminary experiments nicotine dissolved in the saline solution was applied in similar manner.

RESULTS

Some of the mechanocardiograms obtained by this method are illustrated in figures 3 to 7. Figure 3 shows the form of the record when the site of the lever attachment is the third abdominal segment of the isolated heart preparation. The time markings appear as vertical lines cutting across the entire width of the record, and denote intervals of 1 second. In this, as in all the records illustrated, the

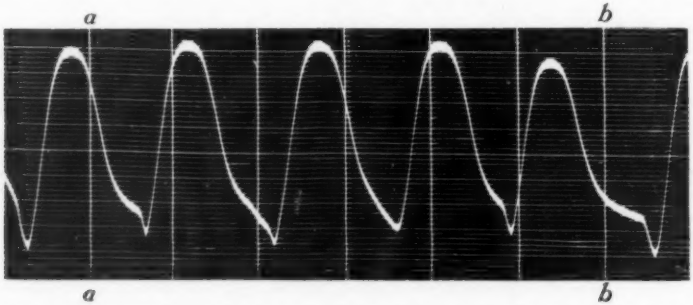

FIGURE 3.—A mechanocardiogram obtained from the third abdominal segment of an isolated heart preparation from *Periplaneta americana*. A rise of the curve represents a dorsally directed movement and a fall of the curve represents a ventrally directed movement of the ventral wall of the heart and the adjacent dorsal diaphragm. The vertical lines cutting entirely across the record are time markings denoting intervals of 1 second. The horizontal lines, made by the usual ruling behind the slit of the electrocardiographic camera, can be used to calibrate in terms of contraction amplitude. The record is to be read from left to right. Distance from line *aa* to line *bb* on original mechanocardiogram is 112 mm.

upward trend of the curve represents a downward (dorsally directed) movement of the dorsal diaphragm at its point of attachment to the ventral wall of the heart (i. e., at the site of attachment of lens paper, fig. 1, *lp*, to cardiac mechanism), while the downward trend of the curve represents an upward (ventrally directed) movement of the same region. Figure 4 shows a typical record obtained from the midabdominal region of the isolated heart preparation and analyzed into its component regions. Under the ordinary conditions of heartbeat and under the conditions of these experiments, the insect cardiac cycle in the midabdominal region of the heart consists of a systolic period (*sy*), represented in the mechanocardiogram by the marked sudden rise of the curve; a diastolic period (*dl*), represented by the marked sudden fall of the curve; and a diastatic period (*dt*), represented by the approximately horizontal region of the curve preceding the succeeding contraction. Often a sudden fall of the curve immediately precedes the systolic rise, appearing as a "presystolic notch." It can be seen also that the diastolic fall of the curve occurs in two fairly distinguishable stages, the first (*1dl*) including the earlier, sudden down stroke of relaxation, and the second (*2dl*) including the

later, less rapid downward trend, which finally becomes the diastatic region.

The mechanocardiogram can be obtained from a single isolated segment, separated from the rest of the isolated heart preparation by complete transverse sections made immediately in front of and immediately behind the required segment. Examples of such records are shown in figure 5. In general, the form of the curve is about the

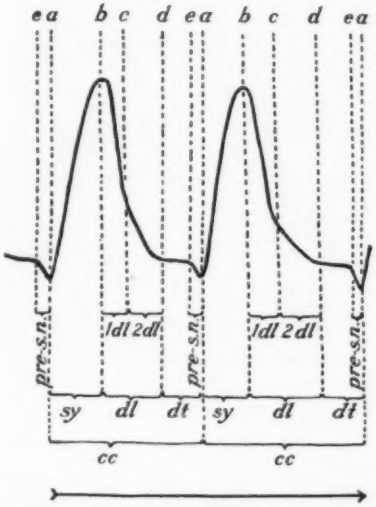

FIGURE 4.—A diagram representing two complete cardiac cycles, analyzed into their component parts as indicated by the vertical broken lines and by the lower braces. The curve is to be read from left to right, as indicated by the arrow: *cc*, Cardiac cycle; *sy*, systole; *dl*, diastole; *dt*, diastasis; *pre-s. n.*, presystolic notch; *1dl*, first part of diastole; *2dl*, second part of diastole; *a* . . . . *e*, lettering to distinguish vertical broken lines.

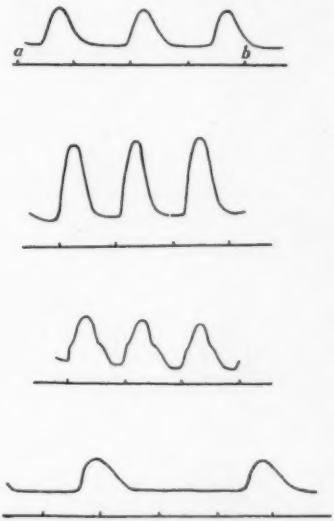

FIGURE 5.—Tracings of mechanocardiograms obtained from completely isolated single abdominal segments separated by transection from other segments of the isolated heart preparation. Time intervals of 1 second are indicated below each curve, which is to be read from left to right. Distance from point *a* to point *b* (top curve) on original mechanocardiogram is 66 mm.

same as that shown in figure 3, except that usually the presystolic notch is lacking.

Mechanocardiograms obtained from various segments of the isolated heart preparation are shown in figure 6. In this figure, 3*th* is obtained from the third thoracic segment, 1*abd* to 6*abd* from the first to sixth abdominal segments, and 7+8 *abd* from the seventh and eighth abdominal segments. Since these records were obtained with the same lever arrangement, the magnification is approximately the same for all. It can be seen that the amplitude tends to be greatest in the region of the fourth abdominal segment.

The effects produced in the mechanocardiogram by the application of nicotine to the isolated heart preparation are shown in figure 7. "Normal" represents the mechanocardiogram obtained from the nonnicotinized isolated heart preparation bathed with physiological saline solution. This record, like the others of this figure, was taken

with a lower camera speed than those of the preceding figures. The curves "Nicotine 1" to "Nicotine 3," inclusive, represent records made at successive time intervals after the application of nicotine to the heart preparation. The curves "Recovery 1" to "Recovery 4," inclusive, represent successive stages of recovery after the nicotinized preparation had been washed with fresh physiological saline solution. As the nicotine acts upon the cardiac mechanism, the mechanocardio-

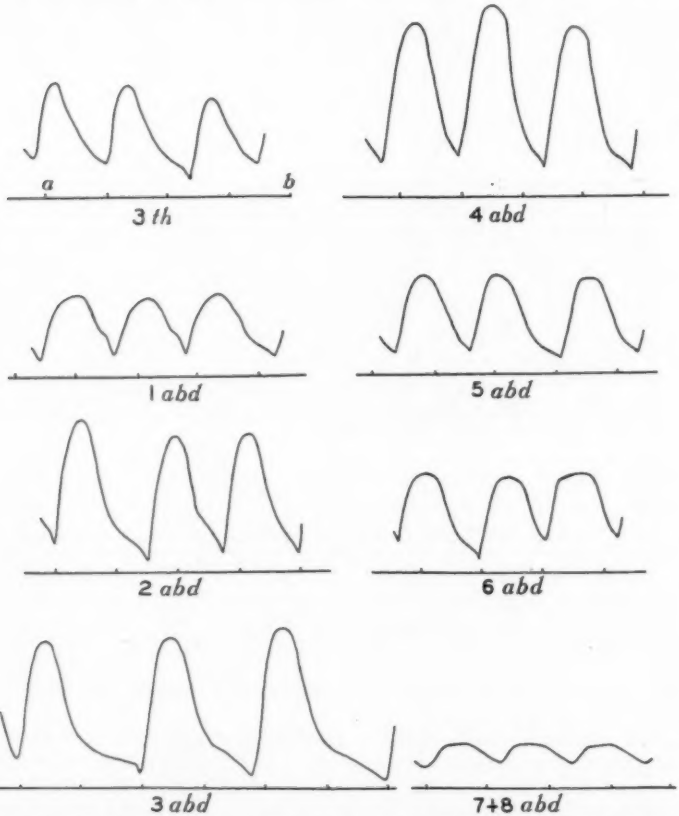

FIGURE 6.—Tracings of mechanocardiograms obtained from various segments of the isolated heart preparation, as indicated by the following labeling: *3th*, Third thoracic segment; 1*abd*, first abdominal segment; and so on through the sixth abdominal segment; 7+8 *abd*, seventh and eighth abdominal segments considered together. The curves are to be read from left to right. Time intervals of 1 second are indicated below each curve. Distance from point *a* to point *b* (*3th*) on original mechanocardiogram is 75.5 mm.

gram shows increasing irregularity of amplitude. Some irregularity of contraction height occurs, but a greater irregularity appears in the degree of diastolic fall of the curve; as the nicotine effect increases, the diastolic fall becomes less and less, until eventually the heart approaches systolic arrest. During recovery relaxation becomes more complete, marked irregularities of contraction height persist for

a shorter or longer time, and premature and partially summated contractions tend to persist for a relatively longer time; eventually, however, the form of the mechanocardiogram approaches that of the heart preparation preceding nicotinization (Recovery 4). With

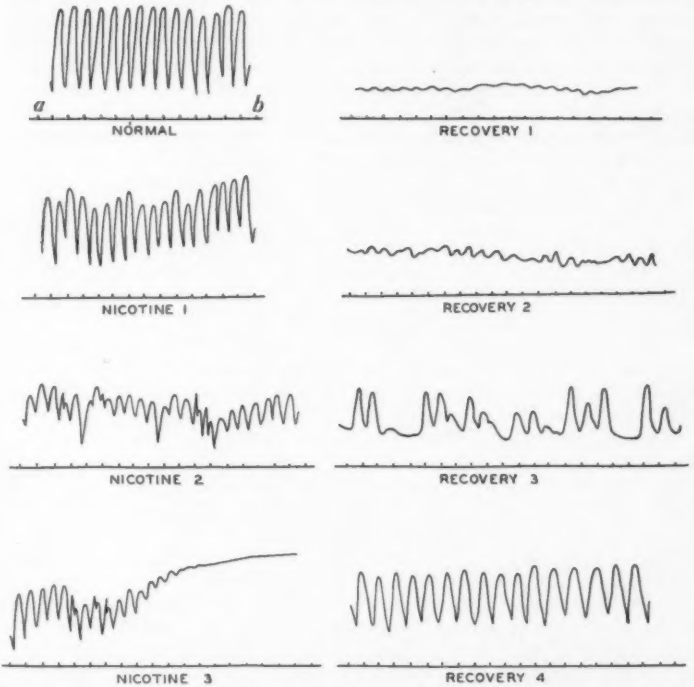

FIGURE 7.—Tracings of mechanocardiograms showing the effect of nicotinization of the isolated heart preparation. The curves, to be read from left to right, are from a single heart preparation. Normal tracing was made before nicotinization. Nicotine 1, 2, and 3 are tracings obtained at successive time intervals after application of nicotine. Recovery 1, 2, 3, and 4 are tracings made at successive time intervals after the heart preparation was washed with fresh saline solution. Distance from point *a* to point *b* (Normal) on the original mechanocardiogram is 74 mm. Time intervals of 1 second are indicated below each curve.

continued washing of the nicotinized tissue with fresh saline solution, the original form of the mechanocardiogram can be restored, provided the nicotinization has been neither too intense nor too prolonged.

DISCUSSION

The idea of utilizing a hair to transmit insect cardiac contractions to a small lever and photographing the lever movement has been applied by Kojantchikov (*2*) in a study of the heartbeat of the cockroach (*Periplaneta*) *Blatta orientalis* L. Kojantchikov's lever made contact with the heart by means of a piece of fat body tied to the free end of the hair, and, depending entirely upon the lever for magnification of heart movement, made a photographic tracing with a kymograph camera. The mechanocardiograms thus obtained consisted of only slight fluctuations of the curve, the highest magnification

being about three times. Uramoto (*5*) obtained smoke-drum (kymograph) tracings of the heartbeat of the silkworm larva by means of a light straw lever connected with the heart (isolated) by means of cellophane or resting upon the dorsal wall of the intact insect. Since, in making these records, magnification of heart movement was by means of lever only, the fluctuations in the kymograph tracings were small. In the method described here both magnification by lever and magnification by an optical projector system have been utilized, with the result that mechanocardiographic curves of sufficient size to permit analysis have been obtained. Most of the records referred to in this paper were made with magnifications of 400 to 600, but much greater magnifications can be obtained when desired. The factors determining the desired magnification are the width of the bromide paper, the intensity of the illumination, and the size and distinctness of the edge of the lever shadow. Although mechanocardiograms obtainable by this method are superior to those published by Kojantchikov (*2*) and Uramoto (*5*) and are more easily subjected to analysis, these investigators were the first, insofar as the author is aware, to obtain mechanocardiograms of the insect heartbeat.

From figure 6 it can be seen that the relative amplitude and the detailed form of the mechanocardiogram vary with the site of the lever attachment. Usually the largest relative amplitude is from the midabdominal region (fourth or fifth abdominal segment).

The significance of the presystolic notch is not entirely clear. Theoretically it should result from any presystolic force or forces that produce a ventrad movement of the ventral wall of the heart or of the adjacent region of the dorsal diaphragm. From a consideration of the anatomical relationships of the dorsal diaphragm to the cardiac tube in the American cockroach, it becomes apparent that the presystolic notch may be the result of alary muscle contraction, especially of those alary muscle fibers that lie approximately in the plane of the dorsal diaphragm. It is also obvious that the presystolic notch may result from a passive dilation of the cardiac tube by an increased pressure of intracardiac fluid resulting from a contraction wave occurring in another region of the heart than that producing the mechanocardiogram. Some evidence has been obtained that the presystolic notch can result from increased intracardiac pressure produced in the manner just suggested. No evidence has yet been obtained as to whether the presystolic notch may also be caused by alary muscle contraction. The correct interpretation of the presystolic notch must await further investigation.

In these experiments, as in those of Kojantchikov (*2*), it has been observed that as the rate of heartbeat increases the length of the diastatic period decreases until, with a rapid heartbeat, it may entirely disappear, in which case each systole begins at some point in the last portion of the diastolic period.

At a given moment the effect of nicotine upon amplitude may be relatively independent of its effect upon rate of contraction. In figure 7, Nicotine 1 and Nicotine 2, the mechanocardiograms were obtained at times during the onset of the nicotine effect when the rates of beat were not very different from that of the preparation before nicotinization; yet the contraction amplitude is markedly affected, especially with respect to relaxation. The cardiac mechanism tends to remain in a systolic condition and eventually (Nico-

tine 3) comes to systolic arrest. This observation is in agreement with the conclusion of Burridge (*1*) and Langley (*3*) that nicotine tends to decrease the ability of striated muscle to recover from the contraction state.

The method described here is applicable not only to the cockroach heart but also to the hearts of many other insects, provided the size and form of lever and its hair attachment to the heart are adapted to the particular species of insect employed. The method can also be used to record movements of various parts of the alimentary tract of the dissected preparation and to study the effects of pharmacological and toxicological substances upon various regions of the alimentary tract and other mechanically active portions of the insect body.

SUMMARY AND CONCLUSIONS

With the photographic method described in this paper, records (mechanocardiograms) of the insect heartbeat can be obtained of sufficient size and clarity to serve for analysis of the cardiac mechanism and the effects of various factors acting upon it, including the effects of various pharmacological and toxicological substances; it can be similarly used with respect to the alimentary tract or other mechanically active portion of the insect body.

Both rate and amplitude of insect heartbeat in the isolated preparation can be simultaneously and quantitatively measured by this method.

From the preliminary experiments reported here, the following conclusions have been reached with regard to the isolated heart preparation of the cockroach *Periplaneta americana* (L.):

The detailed form of the mechanocardiogram varies with the site of attachment of the lever to the heart, but in general, with a low rate of beat, indicates a cardiac cycle consisting of systole, diastole, and diastasis.

As the rate of beat increases, the diastatic (rest) period tends to become shorter and, with a sufficiently high contraction rate, may disappear.

Usually the systolic rise of the mechanocardiographic curve is preceded by a marked sudden fall, for which the name "presystolic notch" is suggested.

The significance of the presystolic notch is not completely apparent, but evidence has been obtained that it can result from increased intracardiac pressure.

Nicotine can markedly affect amplitude of beat with relatively little simultaneous effect upon contraction rate.

Nicotine appears to decrease the ability of the heart to relax, with the result that it may be brought to systolic arrest.

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THE TOXICITY OF PHENOTHIAZINE AND SOME OF ITS OXIDATION PRODUCTS, IN EXPERIMENTS WITH CARASSIUS AURATUS [1]

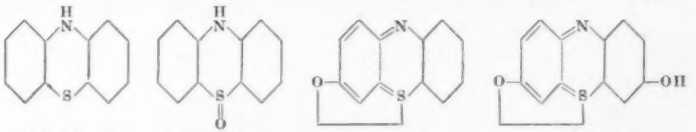
By W. A. GERSDORFF, *associate chemist*, and H. V. CLABORN, formerly *junior chemist, Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

Recently it was reported [2] that, in the course of pharmacological experiments on rats with a newly proposed insecticide, phenothiazine (*2*, *8*),[3] evidence had been found in the feces of the rats of a colored derivative of this substance that possessed strong bactericidal properties. This suggested the possibility of the existence of toxic oxidation products of phenothiazine. Unpublished results of tests for insecticidal action, however, with two such products, phenothiazone and phenothiazine sulphoxide, made by E. H. Siegler, of the Division of Fruit Insect Investigations, against larvae of the codling moth, and by D. E. Fink, of the Division of Control Investigations, against mosquito larvae, have indicated little, if any, toxicity of these compounds to these insects in comparison with that of phenothiazine.

A study of this subject was undertaken by the authors with a method in which the goldfish (*Carassius auratus*) is used as a test animal. Because the goldfish is very susceptible to certain types of poisons, relatively low concentrations are permitted, thus increasing the chances of the tests being made within the range of solubility.

The substances studied were phenothiazine, phenothiazine sulphoxide, phenothiazone, and thionol. For comparative purposes a series of tests was also made with rotenone. The relationships of phenothiazine and its derivatives are shown by their structural formulas.



Phenothiazine — Phenothiazine sulphoxide — Phenothiazone — Thionol

Phenothiazine was prepared by the method described by Smith and his coworkers (*8*). One mole of diphenylamine and two moles of sulphur were fused at 180° C. with the use of iodine as a catalyst. The reaction product recrystallized from toluene gave the pure phenothiazine, a light yellow crystalline compound melting at 180° C.

Phenothiazine sulphoxide was prepared by the oxidation of phenothiazine with hydrogen peroxide as described by Pummerer and Gassner (*7*). It is a slightly yellow crystalline compound which melts at 250° C.

[1] Received for publication Nov. 13, 1937; issued April 1938.

[2] By Floyd De Eds, J. O. Thomas, and C. W. Eddy, of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, and A. B. Stockton, of Stanford University Medical School, at the meeting of the American Society for Pharmacology and Experimental Therapeutics at Washington, D. C., on March 26, 1936.

[3] Reference is made by number (italic) to Literature Cited, p. 282.

Phenothiazone is a purplish red crystalline compound melting at 162° C. It was prepared by the oxidation of phenothiazine with anhydrous ferric chloride by the method described by Pummerer and Gassner (*7*).

Thionol was prepared by treating thionin with sulphuric acid according to a method described by Bernthsen (*1*), although it was found necessary to decrease the sulphuric acid to 65 percent. The compound is a greenish-black crystalline powder having a metallic luster. It does not melt sharply; but, since it is soluble in both concentrated hydrochloric acid and ammonium hydroxide and gives all the color tests reported by Bernthsen, there is no doubt as to its identity.

EXPERIMENTAL PROCEDURE

The method of making the toxicological tests was essentially the same as that described in a previous paper (*3*). Goldfishes of a single lot were used, and a constant temperature of 27° $\pm$0.2° C. was maintained. The fishes weighed approximately 5 to 7 g.

Except in the case of phenothiazone, acetone was used as a dispersive agent, stock solutions of the substances being prepared with it, from which the proper aliquots were taken for the tests. In the making of the test solutions, a higher concentration of acetone than 1 cc per liter of water, which is a concentration nontoxic to goldfishes weighing 2 g or more, was avoided. Phenothiazine and phenothiazine sulphoxide are so insoluble in water and, possibly only on that account, so slightly toxic that in the high concentrations needed they precipitated quickly. Thionol is but slightly soluble in acetone and alcohol (which could have been used in place of acetone) as well as in water, and the acetone used in the tests was little more than a wetting agent. As phenothiazone is soluble in water at the concentrations used, this solvent was used in preparing stock solutions. Acetone was used, as is customary, in the preparation of the test solutions of rotenone; the aqueous solution, however, was apparently complete at the highest concentration used.

RESULTS

The toxicological data are given in table 1. It is at once apparent that phenothiazone, the only one of the sulphur compounds readily soluble in water, has a comparatively high toxicity to goldfish of this size. The toxicity of this substance as compared with that of rotenone is seen from the survival-time curves of figure 1 and the velocity-of-fatality curves of figure 2. Curves are also drawn for phenothiazine, but it must be emphasized that they are only an indication of toxicity under the conditions of suspension.

The frequency distributions of the observed survival times for both rotenone and phenothiazone do not have the pronounced skewness characteristic of phenolic compounds previously studied (*5*, *6*), which led to the use of geometric rather than arithmetic means. Those of rotenone, however, are much more skewed than usual, seemingly because of the much greater resistance of these larger goldfish to this compound. In view of this, and also to make possible ready comparison with recent work, geometric means were again used in drawing the graphs and in expressing relative toxicity.

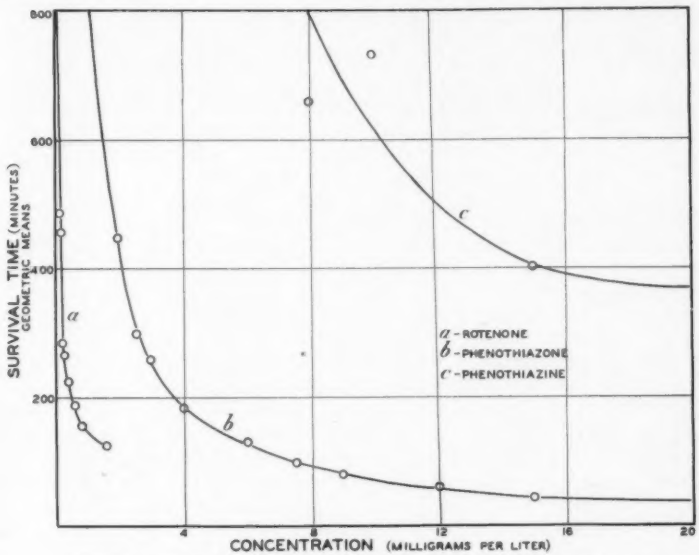


FIGURE 1.—Survival-time curves for phenothiazone, rotenone, and phenothiazine.

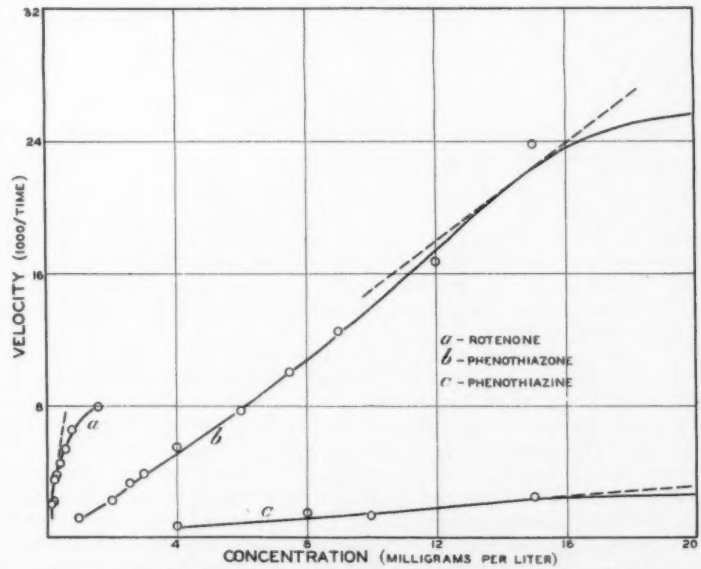


FIGURE 2.—Velocity-of-fatality curves for phenothiazone, rotenone, and phenothiazine.

TABLE 1.—*Toxicity of phenothiazine and its oxidation derivatives and of rotenone to goldfish at 27.0° ±0.2° C.*

Compound and concentration [1] (milligrams per liter)	Fishes used	Mean length of fishes	Mean weight of fishes [2]	Mean survival time [3]		1,000÷geometric mean survival time (velocity of fatality)
				Arithmetic	Geometric	
	Number	*Millimeters*	*Grams*	*Minutes*	*Minutes*	
Phenothiazine:						
50.0	2	65	7.5	ca. 360	ca. 360	2.78
15.0	9	[4]	[4]	>450	>400	<2.50
10.0	10	61	6.6	860±110	730 × 1.18	1.37
8.0	8	57	5.5	730±100	660 ÷ 1.13	1.52
4.0	8	[4]	[4]	>1,500	>1,500	<.67
1.0	12	[4]	[4]	[5] (48 hours)		
Phenothiazine sulphoxide:						
50.0	3	[4]	[4]	(27 hours)		
10.0	3	[4]	[4]	(48 hours)		
Thionol:						
50.0	3	[4]	[4]	(23 hours)		
10.0	10	[4]	[4]	(30 hours)		
1.67	10	[4]	[4]	(30 hours)		
Phenothiazone:						
50.0	2	59	6.0	40±3	40 × 1.07	25.0
15.0	10	59	6.0	43±3	42 × 1.07	23.8
12.0	9	56	5.3	61±2	60 × 1.04	16.7
9.00	10	58	5.8	82±3	80 × 1.04	12.5
7.50	10	57	5.4	101±3	100 × 1.03	10.0
6.00	10	59	6.0	131±3	130 × 1.03	7.69
4.00	11	56	5.2	183±3	182 ÷ 1.02	5.49
3.00	10	58	5.7	259±7	257 × 1.03	3.89
2.57	9	60	6.3	307±17	299 × 1.05	3.34
2.00	12	59	6.0	480±38	446 × 1.09	2.24
1.00	12	58	5.7	940±77	874 × 1.08	1.14
Rotenone:						
1.600	13	62	6.8	130±6	127 × 1.04	7.87
0.800	19	57	5.5	157±5	155 × 1.03	6.45
0.600	25	58	5.8	202±10	187 × 1.06	5.35
0.400	17	58	5.8	239±15	223 × 1.07	4.48
0.300	34	58	5.6	283±12	266 ÷ 1.04	3.76
0.225	6	57	5.3	306±39	282 × 1.14	3.55
0.200	10	62	6.8	480±35	457 × 1.07	2.19
0.150	6	57	5.5	521±63	486 × 1.14	2.06
0.075	8	57	5.3	>700	>700	<1.43
0.050	12	[4]	[4]	(31 hours)		

[1] As the first three compounds are insoluble, the figures signify the quantity added to the water; the last two compounds form true solutions.
[2] Estimated from length, which measurement excludes the tail.
[3] The limits of error indicated are probable errors of the means.
[4] Fishes not measured or weighed but of same approximate size.
[5] Figures in parentheses indicate that the material was not lethal in the time indicated.

Since in this case the two types of curves have reciprocal relationship, interpolation was possible. Therefore, the velocity-of-fatality curve was drawn from selected points on the more readily drawn survival-time curve, especially in the neighborhood of a critical point to be discussed later.

The test suspension of phenothiazine of 50 mg per liter, after exposure to the air and light for several days, acquired a pinkish color similar to that of phenothiazone at a concentration of about 1 mg per liter. So slight an increase in a toxic effect already but poorly defined could not be demonstrated with the small number of fishes permitted.

The test suspension of phenothiazine sulphoxide of the same concentration acquired in a few days a deeper color similar to that of phenothiazone at a concentration of 4 to 5 mg per liter. In the same time appreciable toxicity had developed, two fishes being killed in 140 minutes. Just before this test the suspension was aerated for half an hour, to avoid any toxic effect due to depletion of oxygen. During the test there was no evidence of oxygen hunger on the part of the fishes.

It therefore appears that, whereas phenothiazine sulphoxide may change appreciably on exposure to air to a compound coloring solutions similarly to phenothiazone and about as toxic, such a change in phenothiazine is slight. Within an organism, however, conditions for oxidation may be better than in this simple test, the toxic effect being limited only, as in the case of the goldfish, by the "invadability" of the compound.

DISCUSSION OF RESULTS

It is apparent at once from the curves in figures 1 and 2 that phenothiazone is appreciably less toxic than rotenone. The quantitative ratio depends somewhat on the method used in making the comparison, as emphasized in previous papers (*4*, *6*). A method that compares toxicity in the range of most efficient action is the comparison of the minimum products of concentration and survival time (*4*). These values are calculated directly from the survival-time curves. They may, however, be determined geometrically from the velocity-of-fatality curves by drawing the maximum tangents to the curves from the origin, as indicated in figure 2. In the latter case the slope of these lines, expressed in the units of the two variables, will give the reciprocals of the minimum products. The data for comparison by this measure are given in table 2. The minimum product of concentration and survival time is designated by $c_m t_m$ and, since toxicity varies inversely with this value, its reciprocal is also given. Its approximate coordinates c_m and t_m are given so that the region of the curve fulfilling this condition may be located readily.

TABLE 2.—*Relative toxicity to goldfish at 27° C. of phenothiazine, phenothiazone, and rotenone*

Compound	c_m	t_m	$c_m t_m$	Toxicity, $\frac{1}{c_m t_m}$	Relative toxicity
	Milligrams per liter	*Minutes*	*Gram-minutes per liter*	*Liters per gram per minute*	
Rotenone	0.26	300	0.078	13	1.0
Phenothiazone	14	48	.67	1.5	.11
Phenothiazine	12	500	6.0	.17	.013

These results show that, when compared at the range of most efficient toxic action to goldfish of this size at 27° C., rotenone is nearly 10 times as toxic as phenothiazone, and the latter is approximately 10 times as toxic as phenothiazine. It must be stressed, however, that the phenothiazine is not wholly in solution, and because of the wide distribution of results the values give only an indication of the relative toxicity under these conditions of suspension. Likewise, the insolubility of phenothiazine sulphoxide and thionol may preclude any toxic action under these conditions.

The fact is significant that, on standing, the test suspensions of phenothiazine and phenothiazine sulphoxide acquired a pinkish color similar to that of phenothiazone solutions, accompanied by an increase in toxicity. This change was much the greater in the case of the sulphoxide. In addition such suspensions, as well as the filtered colored solutions, showed a delayed toxic action, as indicated by the fact that fishes taken from incomplete tests in an active upright condition

and placed in fresh water later died. It therefore appears likely that these two compounds may oxidize to a more toxic compound, which may be phenothiazone.

SUMMARY

A study was made of the toxicity with respect to concentration and survival time at 27° C. of phenothiazine, phenothiazine sulphoxide, phenothiazone, and thionol, and the results were compared with those for rotenone. Goldfishes of the same lot weighing approximately from 5 to 7 g were used as the test animals.

Phenothiazone dissolved completely, the other sulphur compounds but slightly, in water at the concentrations required.

The most toxic of the sulphur compounds under the conditions of the comparison was phenothiazone. According to the minimum product of concentration and survival time, which measures toxicity at its range of most powerful action with respect to these two variables, phenothiazone was one-tenth as toxic as rotenone, but was itself 10 times as toxic as phenothiazine.

Phenothiazine sulphoxide and thionol were not appreciably toxic to goldfish of this size.

Test suspensions of phenothiazine sulphoxide acquired appreciable toxicity when exposed to the air for several days and took on a reddish color similar to that of solutions of phenothiazone. Test suspensions of phenothiazine went through a similar change but to a lesser degree.

It appears probable, therefore, that the toxicity of phenothiazine to goldfish may be due to oxidation, after absorption through the gills, to a more toxic compound, which may be phenothiazone. In that case the toxic effect is small because of the insolubility of phenothiazine.

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A PORTABLE INSTRUMENT FOR THE ANALYSIS OF HYDROCYANIC ACID GAS-AIR MIXTURES[1]

By R. J. WILMOT, *specialist, Fumigation Research, Florida Agricultural Experiment Station*, and T. N. GAUTIER, *instructor in physical science, University of Florida*[2]

INTRODUCTION

From the inception of fumigation research in Florida, it has been felt that an apparatus giving instantaneous concentration readings of various fumigants during the fumigation process would be a boon to the worker in this field. Fumigation experiments have revealed that most of the harmful agricultural insect pests succumb to very low concentrations of hydrocyanic acid gas in air. The analyzer for use in studying the effects of HCN as an insecticide must, therefore, measure low concentrations of the gas (less than 1 percent) to within a few hundredths of a percent. A search of the literature failed to locate an instrument of this sort until mention of a simple device for measuring concentrations of HCN in air was found in a paper by Peters.[3] Details of this instrument were not given in the paper and subsequent correspondence brought word from the author that the apparatus was not practical. In the face of this, however, work was started on the apparatus to be described.

The instrument depends for its operation upon the presence of a combustible gas mixed with air, passing through a "cell" containing an electrically heated platinum wire. If the current in the wire and the flow of the gas mixture through the cell are kept constant, any combustion of the gaseous mixture taking place at the surface of the platinum wire will cause a definite increase in the resistance of the wire which can be detected by means of a Wheatstone bridge.

THE CELL

It was found by experiment, using a potentiometer, that No. 36 platinum wire was the smallest gage filament that could be used and maintain consistent results, and that a heating current of approximately 1.4 amperes was required to heat the wire to the combustion point of the gas mixture. At this temperature the wire glows a dull red. Accordingly the cell was designed to carry 7 cm of No. 36 platinum wire giving approximately 2.5 volts drop when carrying 1.4 amperes of current.

Several shapes and designs of cells were tried before the one shown in figure 1 and described below was adopted. In this cell the heated filament is placed directly in the flow of gas; this is made necessary by the low concentrations of HCN used, and requires that the flow of gases be kept sensibly constant. The arrangement has the advan-

[1] Received for publication August 10, 1937; issued April, 1938.
[2] The writers acknowledge with gratitude the valuable assistance in the design of the electrical circuit rendered by L. W. Gaddum, biochemist, Florida Agricultural Experiment Station, and R. C. Williamson, professor of physics, University of Florida.
[3] PETERS, GERHARD. A SHORT GUIDE TO TREE FUMIGATION. [Transl. by M. Herbert.] Ed. 2, 191 pp., illus. Frankfort o. M. 1934.

tage, however, that no bulky cooling baths for the cell are required; the passage of the gas mixture through the cell carries away excess heat. To facilitate this cooling action, several turns of the copper tubing leading the gas mixture to the cell are wound around the cell and the unit is wrapped in asbestos paper to eliminate the effects of air currents.

The filament is zigzagged across the cell and welded to two lead-in wires of nickel-chromium alloy which are, in turn, soldered into two brass bolts passing into the cell through airtight bakelite sleeves. This end of the cell is threaded into the other half so that the filament is easily exposed if repairs are necessary.

The above-described cell departs somewhat from the type used in the Mine Safety Appliances Co.'s combustible gas indicator, and that of the Leeds & Northrup CO_2 recorder.[4]

GAS CIRCULATION

As mentioned above, the flow of gas past the filament must be maintained at a constant value, and in fumigation it is also necessary to draw the gas from some distance because it is unsafe for the operator to enter the space being fumigated. A flow of 1 liter per minute was found to give satisfactory sensitivity and steadiness of operation. To supply this circulation a positive rotary pump was constructed. Driven by a small 6-volt motor, the pump is capable of drawing a sample from a distance of over 100 feet through a ¼-inch copper tube at the rate of 1 liter per minute. The motor under normal load uses approximately 1½ amperes of current.

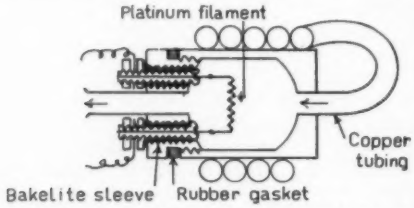

FIGURE 1.—Cell with heated platinum filament through which the gas flows.

It can be seen that the use of this instrument in connection with experimental work with fumigatoria is limited to those of the larger size because of the fact that it is necessary for the sampling flow to be continuous. At least, it should be to give satisfactory results. In a 100-cubic foot fumigatorium, however, the concentration would only be reduced six points in the third decimal place with a theoretical concentration of 0.3 percent HCN by volume. The instrument has been used in connection with a 20-cubic-foot box [5] with a flow of one-half liter per minute and it was calibrated accordingly. In this case there was no leakage and the volume decrease could be compensated for.

The sampling tube is connected to the instrument by means of a brass copper-tubing connection fastened into the instrument panel. This connector is drilled and tapped out to hold a small brass tube, one end of which is covered with fine copper screen to act as a flash-back arrestor. From this connector a copper tube carries the sample

[4] ROSECRANS, CRANDALL Z. AUTOMATIC GAS ANALYSIS RECORDER FOR THE RANGE OF 0 TO 3.5 PERCENT OF CO_2 IN AIR. Jour. Optical Soc. Amer. and Rev. Sci. Instruments 14: 479–490, illus. 1927.

[5] CAMP, A. F., and WILMOT, R. J. FUMIGATION RESEARCH IN FLORIDA. Fla. State Plant Bd. Monthly Bull. (1930–31) 15 (5-8): 1-35, illus. 1931.

to the flowmeter. This flowmeter is an adaptation of the usual type changed in form so that it can be read on a horizontal plane. It is blown from 1 mm capillary pyrex tubing, and in order to have the liquid come to the center or indicator mark on the arm which shows through the bakelite panel, it is necessary to adjust the size of the orifice and the volume of the liquid by trial and error until the correct combination is found (fig. 2). In order to make the orifice adjustable, a short length of copper tubing was flanged on both ends and a disk of platinum foil soldered into one end. The foil is pierced with a hole approximately 0.02 inch in diameter. Platinum was used to obviate corrosion and subsequent change in calibration of the flowmeter. The length of copper tubing is inserted into the flowmeter and is held in place by means of short lengths of rubber tubing, as shown in the diagram. The liquid used is kerosene, which has been treated with sulphuric acid to remove the unsaturated hydrocarbons and then colored with a little gasoline dye. Next to the flowmeter is the needle valve which is used to control the flow. It is a ¼-inch valve with copper tubing connections and a knurled knob replacing the usual T-type handle. From the valve the sample passes through copper and rubber tubing to the cell, then through a HCN gas-mask canister, and from it through a 1-pint expansion chamber to the pump. A small hole is drilled in the last length of tubing to let in extra air to compensate for the excess suction of the pump.

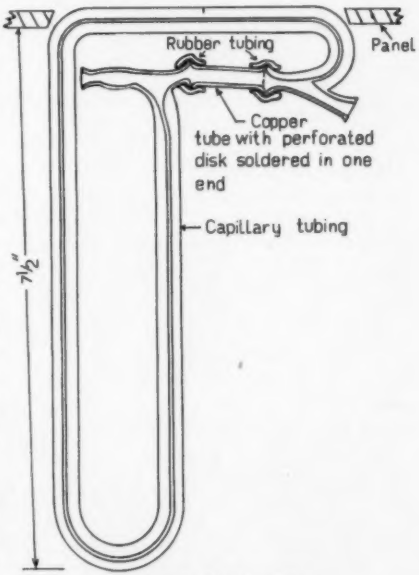

FIGURE 2.—The flowmeter.

The pump (fig. 3) is constructed of brass and steel with a brass rotor and a steel housing which is bolted directly to the motor housing. The rotor is approximately five-eighths of an inch thick and 1¼ inches in diameter, with six vanes set into it. These vanes are of composition similar to Bakelite one-sixteenth of an inch thick and three-eighths of an inch long and are set into the rotor at an angle of 30° to the radius; if rotation is clockwise they should point counterclockwise outward. The housing is bored out one-eighth of an inch off-center so as to be eccentric to the rotor. The inlet and outlet are spaced equally on each side of the point where the rotor and housing touch. A second hole is placed next to the inlet for an oil cup. The oil inlet must be very small, preferably adjustable so the amount of oil entering can be kept to a minimum, and the oil used should be of the lightest grade obtainable.

ELECTRICAL CIRCUIT

In the interest of portability, automatic current compensation and attendant bulky cooling baths have not been used. A 6-volt storage battery is necessary because of the heavy currents used by the bridge and air pump but, in the present-day use of automobiles, one is usually available nearby.

It was found impractical to give each of the bridge arms the resistance values called for by the resistance of the filament. Had this been done heavy current and correspondingly heavy resistance wire would have been necessary in both branches. The final compromise leaves the bridge symmetrical about an axis at right angles to the current and as nearly symmetrical about the other axis as is necessary to give proper sensitivity.

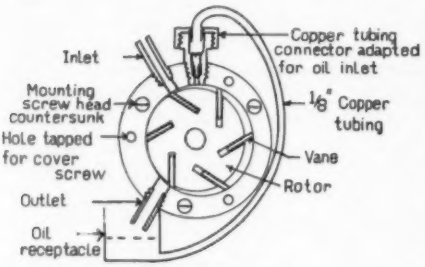

FIGURE 3.—The pump.

The current rheostats *j* (fig. 4) are a 1Ω and a 3Ω in parallel, the 3Ω being used as a fine adjustment on the 1Ω. These rheostats should be of a heavy-duty type capable of dissipating 250 watts under continuous load but should have sufficient windings to give smooth operation. Resistance *xc* is approximately 8 feet of No. 18 resistance wire rated at 0.18Ω per foot with a temperature coefficient of 1.002 for a change of 73° C. This is space-wound on a porcelain tube 1 inch in diameter, 5½ inches long, and is enclosed in an asbestos box. The slide wire *p* (position *dc*, fig. 4) is a 12-inch length of similar resistance wire mounted around the edge of a 4-inch bakelite disk, on the upper side of which is placed the calibrated card seen through a window in the bakelite panel. A shunt, in this case an equal length of the same kind of resistance wire, permits regulation of the effective resistance of the slide wire which, in the present design, is 0.115Ω. Flexible leads connect it to resistance *xc* and the filament *ef*. The brush on the slide wire is of the brass plunger type with a spring to maintain the tension against the wire. A piece of platinum foil is soldered to the end of the plunger to give a good contact surface. The lead from the brush is connected to one corner of a two-pole, double-throw switch (fig. 5, *A*, 6). The adjacent corner of the switch is connected between the 500Ω resistances *l* and *m* (fig. 4). These resistances are wire-wound and will not change with ordinary changes in temperature. The center poles of the switch are connected to a center-zero type galvanometer with a tapping key in the circuit. The galvanometer has a

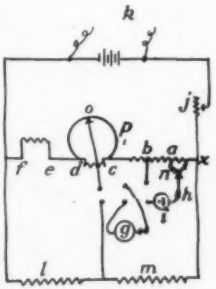

FIGURE 4.—Circuit diagram: Total amperage, 3.0a; filament amperage, 1.4a; resistance *xa*, 0.058Ω; resistance *xb*, 0.936Ω; resistance *xc*, 1.464Ω; resistance *cd*, 0.115Ω, filament *ef*, 7 cm of No. 36 platinum wire; *g*, galvanometer; resistance *h*, 50Ω; *i*, two 1½-volt batteries; resistance *j*, 1Ω and 3Ω rheostats in parallel; *k*, blower switch; resistance *l-m*, 500Ω each; resistance *n*, 200Ω; *o*, slide wire brush; *p*, slide wire.

sensitivity of approximately 2 microamperes per division and a resistance of approximately 125Ω.

The current checking circuit is standard. The span xa in $x+c$ (fig. 4) can be quite small, as its resistance need only be enough to allow com-

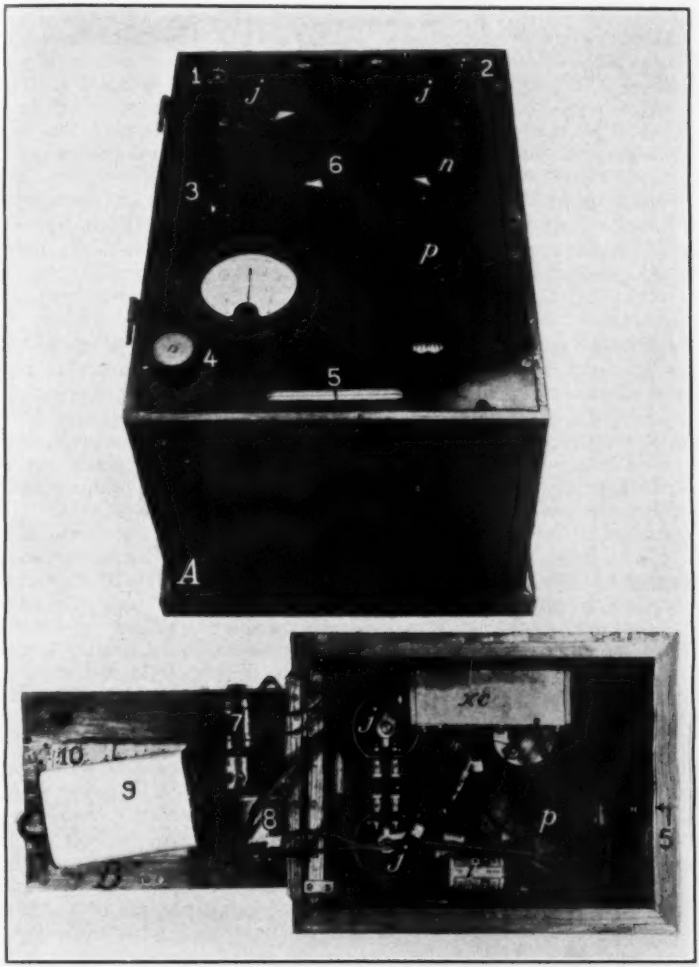

FIGURE 5.—*A*, Exterior of the analyzer: 1, Blower switch; 2, sampling-tube connection; 3, key in galvanometer circuit; 4, needle-valve knob; 5, flowmeter; 6, double-throw double-pole switch. *B*, Interior of the analyzer: 5, Flowmeter; 7, motor and pump; 8, cell; 9, expansion chamber; 10, gas-mask canister. Letters refer to letters on circuit diagram in fig. 4.

pensation for small variations in the zero balance of the circuit which will occur from time to time due to changes in temperature and relative humidity between experiments. The voltage divider shunt should have a resistance large enough to prevent an appreciable

current from flowing through it, and in this case is a 200Ω potentiometer rheostat (fig. 5, *A*, *n*, "CHECK"). It has a power rating of 5 watts. The brush on *p* is connected to the wire-wound resistance *h* (fig. 4), which is a 5-watt 50Ω resistance. The resistance of *h* determines the sensitivity of the check circuit; *h*, in turn, is connected to the positive pole of two 1½-volt flashlight cells (*i*) in parallel. A standard cell could be used at this point for greater accuracy, with corresponding changes in *xa* and *xb* and *h*, but it was thought that the cost was prohibitive. The negative pole of the battery (*i*) is connected to one of the remaining corners of the double-throw switch and the last corner of the switch is connected to the point *b* on resistance *xc*. The value of *xb* is approximately 0.936Ω. It was found that, in determining the proper position of connection *b*, if the battery connections were properly made, that is, with the positive pole of the storage battery to *j*, the positive pole of *i* to *xa*, and the + terminal of the galvanometer to *i*, when the galvanometer needle throws to the left, *b* should be moved toward *c*, and if to the right, toward *x*.

OPERATION OF THE INSTRUMENT

Before the pump is started the bridge is allowed to "warm up." This warming up begins as soon as the battery connections are made and is necessary because of the relatively heavy currents employed. The sampling tube is connected to the instrument and placed in the area to be sampled before the gas is released. After 15 or 20 minutes, when a temperature equilibrium has been reached, the pump may be switched on, and the flow adjusted to 1 liter per minute by opening the needle valve until the liquid in the flowmeter is brought to the indicator mark. With the dial of the slide wire *p* (fig. 5, *A*) set at zero, and the double-throw switch in the "Read" position, the galvanometer is brought to zero by means of the current rheostats *j*. When there is no further change the switch is thrown to the "Check" position and the galvanometer brought to zero by means of the potentiometer rheostat *n* ("CHECK"). After this setting is once made it is not changed during the course of the run, and the current rheostats are used to bring the current to the value determined by the initial "Read" setting with dial at zero.

The HCN is then released and readings are taken at any desired interval directly off the slide-wire dial, first making sure the flow is correct, by throwing the switch to "Check" and bringing the galvanometer to zero by means of the current rheostats, and then throwing the switch back to "Read" and bringing the galvanometer to zero by means of the slide-wire *p*. The reading is then taken off the slide-wire dial. This whole operation can be done in less than a minute. If the concentration is changing rapidly it is best to use the average of two readings. The arrangement of the controls is shown in figure 5.

The sampling tube has to stay in its initial position because the initial settings made were for the particular conditions under which the fumigation was being made and they compensate for the changes that different combinations of temperature and relative humidity might have on the readings. This was thought to be simpler than to calibrate the instrument under standard conditions and then work out correction tables for various combinations. If a number of points in a fumigatorium are to be sampled, there is no reason why the instrument cannot be connected to a manifold and gas be selected from any of

several points by a system of valves. Time would have to be allowed for the tube just coming into use to be thoroughly flushed out with the gas before readings were taken, but with a flow of a liter per minute this would only be momentary.

The instrument cannot be set to zero outside of the space being fumigated unless the operator is sure that the conditions are the same as they are inside. When this is the case, there are no objections to doing so. It is possible that, in orchard fumigation, for instance, the instrument could be moved from tent to tent because the conditions would not change materially from one tent to another.

When the instrument is used in a glass system such as Allison's,[6] having means for measuring and controlling atmospheric pressure, temperature, and relative humidity, the flow is started through the system and the instrument brought to a balance following the usual procedure. The system must be flushed out for some time after use so that there will not be any residual HCN to cause an error in the setting. The flowmeter in such a system can be used to calibrate the flowmeter in the instrument. The flowmeter, pump, gas-mask canister, etc., in the instrument are of course unnecessary when such a system is used because the flow can be maintained by the other means.

CALIBRATION

Calibration readings were taken in two ways: One while the analyzer was connected into a glass system such as is mentioned above, having means for measuring and controlling temperature, relative humidity, and atmospheric pressure, and another in which the sample was drawn from a fumigatorium while it was in operation under actual field conditions. In each case chemical analysis, by a variation of the Liebig method,[5] provided the concentration data used in calibrating the slide-wire settings of the analyzer. It was found that, by having the check circuit adjustable by means of the potentiometer n, a calibration would hold for a temperature range of 10° C. (from 20° to 30°) and a range of relative humidity from 20 percent to 90 percent. The concentration readings obtained with the instrument are accurate to about ± 0.02 percent HCN by volume under field conditions. The calibration was made in percentage by volume since the method of chemical sampling employed volume ratios. Figure 6 is a sample calibration curve in which the chemical analyses have been plotted against readings from an arbitrary linear scale on the slide-wire dial. Using the calibration curve, a concentration scale was marked on a circular card and attached to the slide wire p.

APPLICATION

The instrument described above will analyze hydrocyanic acid gas-air mixtures accurately under most conditions under which fumigation is carried on. It can, therefore, be used to determine whether or not buildings, holds of ships, etc., can be safely entered. It can also be used to determine whether or not the fumigation is being carried on efficiently, because, after all, it is the concentration of the gas reaching the insect that effects the kill.

It is believed that the instrument may be modified for use with other fumigation gases such as carbon bisulphide, since preliminary experi-

[6] ALLISON, JAMES B. STUDIES ON THE TOXICITY OF HYDROCYANIC ACID. Iowa State Coll. Jour. Sci. 2: 243-252, illus. 1928.

ments show a definite response of the instrument to low concentrations of CS_2.

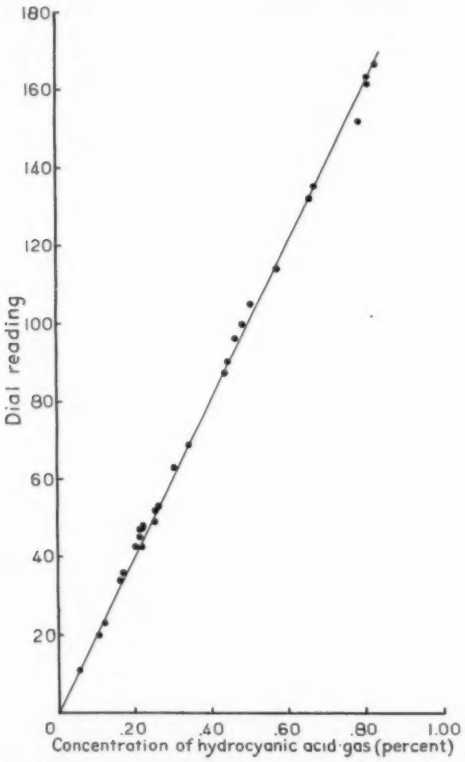

FIGURE 6.—Sample calibration curve in which chemical analyses for HCN were plotted against readings from an arbitrary linear scale on the slide-wire dial.

SUMMARY

An instrument which analyzes hydrocyanic acid gas-air mixtures by physical apparatus is described. The sample is pumped from the space containing the gas by means of a small motor-driven pump contained in the instrument, and passed over a hot platinum filament; the change in resistance of the filament is then measured by means of a bridge, and translated into percentage of HCN by volume. Its accuracy is $\pm$ 0.02 percent HCN by volume.

FUNGUS GROWTH IN SHELLED CORN AS AFFECTED BY MOISTURE[1]

By BENJAMIN KOEHLER

Chief in crop pathology, Department of Agronomy, Illinois Agricultural Experiment Station

INTRODUCTION

Infection of corn caused by ear-rot fungi begins soon after pollination and may continue until stopped either by lack of moisture in the grain or by low temperature. Sometimes these ear-rot fungi cause considerable damage after the corn is cribbed or shelled and placed in storage. The study here reported was made to determine at what moisture limits growth of ear-rot fungi may take place at a temperature suitable for their development. To facilitate study, all the grain used in the experiment was first shelled from the cobs. A number of aspergilli and penicillia became conspicuous in the corn stored under controlled conditions and these were included in the study. These forms have often been recognized as important storage-rot fungi by other workers. They have not been observed by the writer to cause significant losses to corn ears prior to harvest except that they often produce moldy areas where the ears have been injured by birds or insects. Short progress reports of this work have been published (*9*, *12*).[2]

METHODS

STORAGE CHAMBERS

Two styles of storage chambers were used. When no disinfectant was applied because pure culture work was not desired, the shelled corn (*zea mays* L.) was placed in cylindrical wire baskets holding 100 g and hung over salt solutions in wide-mouthed half-gallon glass bottles. In most of the experiments the bottles were supplied with glass tubes 15 cm long with a 3-mm bore which were inserted through the rubber stoppers that closed the bottles. This supplied aeration sufficient to prevent the sour alcoholic odor which developed on corn above a certain moisture content in chambers entirely sealed, and at the same time checked appreciable evaporation. In fact, under sufficiently limited aeration the production of metabolic water by respiration seems to compensate fully for that lost by diffusion.

For pure culture work with a single fungus the apparatus shown in figure 1 was used. This method, adapted from Shippy (*15*, *pp. 370–372*) and Hatfield (*7*), provided a constant humidity and fresh sterile air. Before the surface-sterilized shelled corn, was introduced the entire apparatus was sterilized under steam pressure. Some of the rubber tubes were disconnected during the autoclaving process to prevent the solution from being forced from one flask into another. An additional flask of salt solution at first used in the chain was found

[1] Received for publication July 19, 1937; issued April 1938.

[2] Reference is made by number (italic) to Literature Cited, p. 306.

unnecessary. A battery of 12 of these sets was operated at one time in a constant-temperature chamber. After the first few days of more thorough aeration, the air flow was adjusted to one bubble in 2 or 3 seconds.

HUMIDITY CONTROL

While partial saturations of sulphuric acid are commonly used for humidity control where the relative humidity must be known (*17*, *22*), in this case results were recorded in terms of moisture content of corn, and therefore a series of partial concentrations of any one of a number of salts would answer, and calcium chloride was chosen for use in the half-gallon bottles. A series of concentrations from 24 to 0 percent produced atmospheric humidities which caused the corn moistures to range from 14 to 29 percent.

In the sets where air was bubbled through the solutions, saturated solutions of a variety of chemical compounds were used. The chemicals used are given in table 1. They are arranged in ascending order

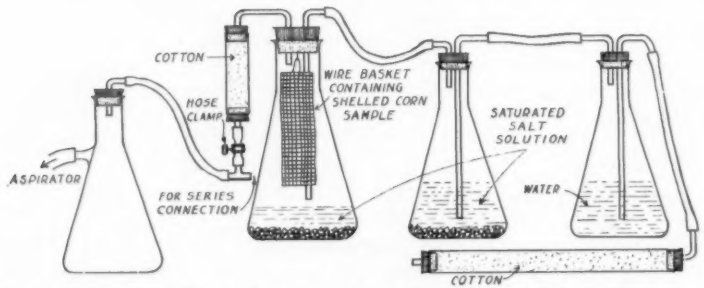

FIGURE 1.—Apparatus used for storing corn under constant-humidity conditions with a slow uniform change of air, and for protecting it from contamination with micro-organisms.

with respect to corn moisture, and therefore should also be in ascending order with respect to relative humidity. The values for relative humidity as compiled by Spencer (*16*) (table 1) are not in full agreement with this. Loss of water from a saturated solution does not affect the relative humidity over the solution. In actual use it was found that after a 3-month run, only a little water need be replenished in the end flask and there was no appreciable change in volume in the flasks containing chemical solutions.

Over some salt solutions, within certain limits, relative humidity is affected very little by temperature, while over others it is affected considerably. Furthermore, in either case a sudden change in temperature would temporarily throw the humidity out of balance, and at high humidities the dew point is easily reached, not only on the inner surface of the flask but also within the corn sample. Therefore, storage was carried out under constant temperature arbitrarily chosen at 70° F.

CORN USED

Well-developed, mature Illinois station Reid Yellow Dent ears free from blemishes were selected and 10 kernels from each were tested on a tray type germinator. This gave an approximation of the kind of internal infection, if any, the ears carried. Disease-free ears and

ears having grain infected with only one fungus, *Diplodia zeae* (Schw.) Lév., *Fusarium moniliforme* Sheldon, *Gibberella zeae* (Schw.) Petch, *Nigrospora sphaerica* (Sacc.) Mason (=*Basisporium gallarum* Moll.), or *Cephalosporium acremonium* Corda were selected. The ears were further selected after surface sterilizing 20 representative kernels from each ear and plating on potato-dextrose agar. Air-dry corn ranging from 10 to 13 percent in moisture and with the desired amount of water subsequently added was usually used, but some checks were made by selecting ears fresh from the field which contained a higher percentage of natural moisture. Whether or not the corn had previously been dried apparently made no difference in the results. This is in agreement with the finding of Swanson (*18*) in work with stored wheat.

TABLE 1.—*Moisture content of unsterilized shelled corn after storing over saturated solutions of different chemicals in closed containers* [1] *for 12 weeks at 70° F.*

Name of chemical	Relative humidity, at 20° C.[2]	Tests made	Moisture content of corn	
			Range	Mean
	Percent	*Number*	*Percent*	*Percent*
Ammonium sulphate	81	4	15.2–16.2	15.7
Potassium bromide	84	6	15.3–17.6	16.5
Potassium hydrogen sulphate	86	13	17.1–19.7	18.2
Zinc sulphate	90	12	17.0–20.7	18.6
Sodium sulphate	95	11	17.9–21.3	18.8
Sodium sulphite	93	15	18.5–22.4	19.9
Barium chloride	88	13	19.0–21.8	20.2
Potassium nitrate	93	12	20.7–22.6	21.6
Sodium tartrate	91	4	21.0–22.2	21.7
Ammonium dihydrogen phosphate	93	9	21.0–23.4	22.2
Sodium bromate	92	16	21.5–23.5	22.3
Dibasic sodium phosphate	95	14	23.2–25.7	24.6
Oxalic acid	96	4	25.1–27.5	26.0
Potassium sulphate	97	7	25.3–27.2	26.2
Gypsum	98	5	27.2–30.1	28.7

[1] As shown in fig. 1. [2] As reported by Spencer (*16*).

The corn moistures were determined by placing about 50 g of the grain in an open vessel 65 mm in diameter and drying at 100° C. for 4 days in an electric oven without vacuum. Percentages are based on weight of corn before drying.

SURFACE STERILIZATION

Where pure culture work was desired, the grain was sterilized on the surface with the filtrate from a fresh chlorinated lime solution. The time of soaking ranged from 15 minutes to 1 hour, depending on how much water it was desired to take up. A solution of 80 g of chlorinated lime per liter of water was used for the short-time soaks, with decreasing strengths for the longer soaks. The grain was treated in an open beaker with frequent stirring and then was transferred to a sterile wire basket and placed in the sterilized storage apparatus. Thorough aeration with sterile air to remove chlorine fumes and thus allow the fungi to develop was provided by the apparatus shown in figure 1.

SOME FACTORS AFFECTING THE AMOUNT OF HYGROSCOPIC MOISTURE TAKEN UP BY GRAIN

VARIABILITY IN CORN STRAINS

Corn from different ears placed over the same solutions did not always come to equilibrium at the same grain moisture content (table 1). An experiment conducted with several distinctly different types of dent corn revealed significant differences in the extent to which water was taken up by the different types from two different atmospheres (table 2). Two cubic centimeters of formalin was added to 198 cc of water in the bottom of the jars to prevent mold growth on the kernels, and the corn used was selected for freedom from internal infection. The formalin solution proved unstable; it gradually lost its disinfecting power and, therefore, was replaced once a month. The largest quantity of moisture was taken up by the old type Reid Yellow Dent corn, which is a moderately rough "starchy" type. The Illinois high- and low-oil and high- and low-protein strains (*10*) differ markedly with respect to oil and protein content. All the hybrids used were of a distinctly horny dent type and the moistures at the close of the test were definitely lower than in either one of the two kinds of Reid Yellow Dent. It was shown by Alberts (*1*) that starchy corn takes up hygroscopic water more rapidly than horny corn. Bailey (*3*) found differences in three varieties of corn with respect to their capacity for taking up hygroscopic water.

TABLE 2.—*Moisture in 10 strains of dent corn when the shelled grain was stored free from mold in two constant atmospheric humidities in sealed half-gallon bottles for 105 days at 70° F.*

Kind of corn	Over 10-percent $CaCl_2$ solution containing 1 percent formalin					Over pure water containing 1 percent formalin				
	Moisture of grain in each of 4 replicates				Average moisture	Moisture of grain in each of 4 replicates				Average moisture
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Station strain Reid Yellow Dent	20.5	20.8	20.0	20.3	20.4	29.0	29.1	28.9	29.1	29.0
Old type Reid Yellow Dent	21.5	21.5	21.0	21.4	21.4	29.9	30.2	30.1	29.7	30.0
Illinois high protein	19.3	19.2	19.3	19.7	19.4	27.8	28.0	27.9	27.7	27.9
Illinois low protein	20.7	20.7	19.7	20.1	20.3	28.9	29.6	27.8	28.5	28.7
Illinois high oil	19.3	19.2	19.0	19.4	19.2	26.8	27.4	27.0	27.3	27.1
Illinois low oil	19.8	20.2	19.6	20.0	19.9	29.4	29.3	29.2	29.5	29.4
Hybrid A5 × Hy [1]	19.9	20.0	19.2	19.4	19.6	27.5	28.0	27.0	27.2	27.4
Hybrid A × K [1]	19.3	19.3	19.3	19.1	19.3	26.5	26.9	26.4	26.4	26.6
Hybrid R126 × JL [1]	19.9	19.9	19.4	19.8	19.8	27.0	27.3	26.5	26.6	26.9
Hybrid (A×L) (Hy×R4) [1]	19.2	19.0	19.7	19.4	19.3	26.7	26.9	26.1	26.8	26.6

[1] Supplied by J. R. Holbert, formerly with U. S. Department of Agriculture.

CONDITION OF GRAIN

The amount of moisture taken up also is influenced considerably by whether the grain is moldy or free from mold (fig. 2). The range or variability in corn moisture over each of the solutions given in table 1 is a combined effect of differences in corn used and differences in moldiness and kind of mold used in the different tests. Alberts (*2*) found that removal of the seed coat from the crown of corn kernels affected the speed with which hygroscopic water was taken up

or lost, but experiments by the writer indicated that this had no effect on the ultimate moisture content of the grain after equilibrium with the surrounding atmosphere was reached, nor did previous killing of the grain by low temperature affect this relationship.

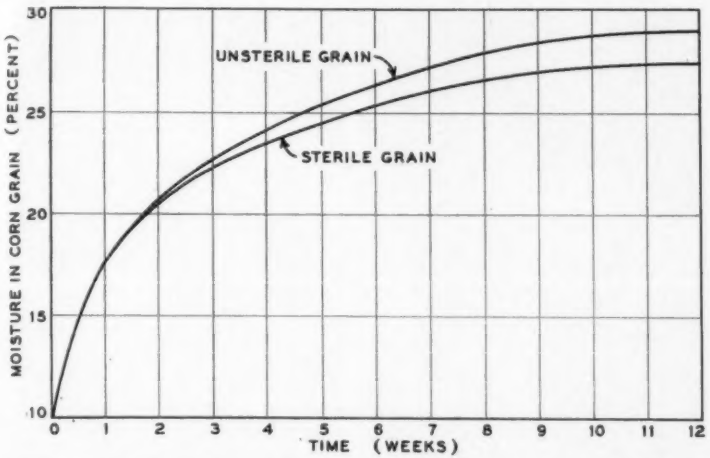

FIGURE 2.—Rise of hygroscopic moisture in shelled corn stored in a saturated atmosphere in closed containers for 12 weeks at 70° F. Grain from the same ears was divided into two lots; one was placed over plain water and molds developed; the other was placed over water containing 1 percent of formalin and remained sterile.

TIME AND TEMPERATURE OF STORAGE

At constant humidity and temperature, corn grain comes to equilibrium with the surrounding atmosphere very slowly, coming close to it in 12 weeks' time at 70° F., as shown in figure 2. To shorten this lag, sufficient water was added to the corn to bring it close to the desired moisture before placing it in the constant-humidity chambers, and a storage period of 12 weeks was chosen. Further fungus growth at low moistures might have been found if the storage period had been longer, for fungus growth is exceedingly slow when near the critical moisture (*18*, *23*). From a practical standpoint, on the other hand, it must be considered that the moisture of corn in storage is seldom static, and when it is above the critical point for mold growth there usually is a tendency for the moisture to decrease slowly.

The temperature chosen, 70° F., also has an important bearing on the results obtained. Swanson (*18*), for instance, found that wheat stored for 13 weeks at 95° F. was safe from mold growth only below 14 percent moisture content, but at 60° it was safe for the same length of time at 17.4 percent.

FUNGUS GROWTH IN RELATION TO MOISTURE OF GRAIN

ASPERGILLUS SPECIES

Members of the *Aspergillus glaucus* group [3] grow at a lower moisture content of grain than any other kind of fungi observed in these

[3] Some isolations of the *A. glaucus* group and *A. versicolor* were identified by Charles Thom, of the Bureau of Plant Industry, U. S. Department of Agriculture.

experiments. Some members of the group produced primarily conidia on the grain as well as on culture media while others produced primarily perithecia, as shown in figure 3, *A* and *B*. The green conidial heads were observed with the naked eye at moistures as low as 14.3

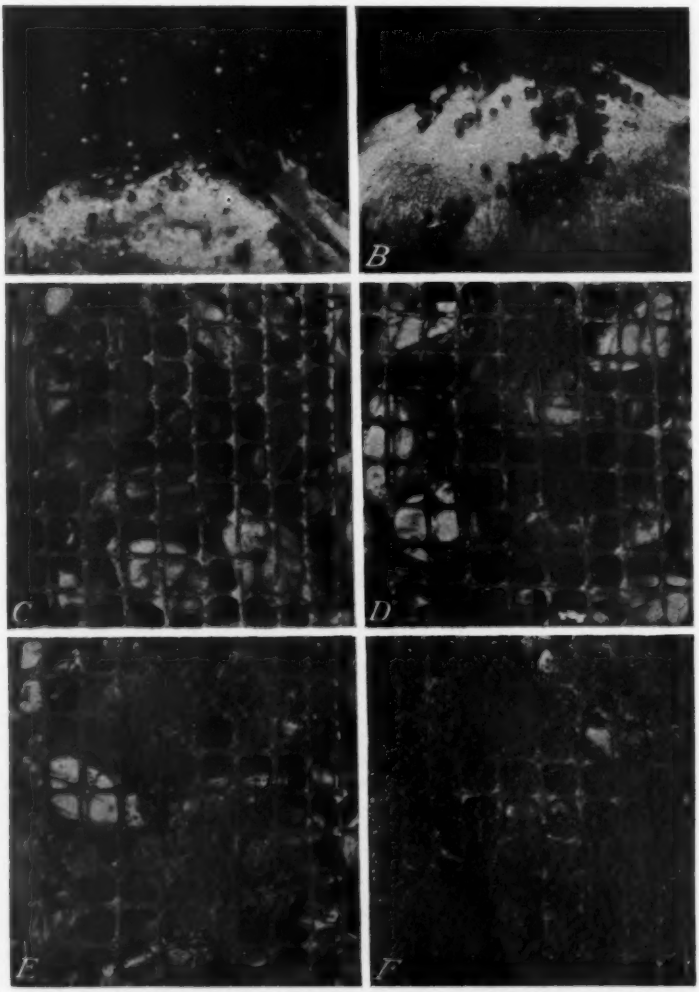

FIGURE 3.—Some aspergilli that developed on corn at low moistures during a 3-month period at 70° F.: *A*, Greenish conidial heads of *Aspergillus glaucus* growing on the tip end of grain with 15.5 percent moisture. *B*, Another form of *A. glaucus* producing bright-yellow perithecia on grain at 16 percent moisture. Both × 15. *A. glaucus* occurred at moistures as low as 14.3 percent. *C*, *D*, *E*, and *F*, *A. wentii* growing at moistures of 15.9, 16.8, 18.0, and 19.2 percent, respectively. × 2.

percent after 12 weeks' storage, as shown in table 3, and bright-yellow perithecia appeared at a moisture percentage only slightly higher. Thom and Le Fevre (*20*) found members of the *A. glaucus* group to

be the first molds active in corn meal as the moisture rose above 12.5 or 13 percent. This was a somewhat lower moisture than that found in the present experiments with whole grain.

TABLE 3.—*Critical moisture content of shelled grain of Reid Yellow Dent corn at which certain fungi were able to grow*

Name of fungus	Source of fungus	Tests made	Highest moisture at which no fungus growth was observed	Lowest moisture at which some fungus growth was observed
		Number	*Percent*	*Percent*
GRAIN NOT SURFACE-STERILIZED, COMPETITION BY OTHER ORGANISMS				
Aspergillus glaucus group	Natural infection	12	14.8	14.4
A. wentii	do	2	15.2	15.1
Penicillium spp	do	9	17.3	16.3
A. flavus	do	3	18.5	18.3
A. niger	do	12	20.8	18.3
Fusarium moniliforme	do	14	21.6	18.8
Diplodia zeae	do	9	22.4	21.2
Gibberella zeae	do	3	22.9	22.2
Nigrospora sphaerica	do	7	24.8	23.6
GRAIN SURFACE-STERILIZED, PURE CULTURE OF FUNGUS PRESENT				
A. glaucus group	Inoculation	2	14.0	14.3
A. versicolor	do	2	14.2	15.0
A. wentii	do	1	14.5	15.4
A. ochraceus	do	2	14.5	15.6
P. notatum	do	3	15.0	15.6
P. viridicatum	do	2	16.8	17.6
P. pallitans	do	3	17.6	18.0
A. flavus	do	2	18.0	18.3
F. moniliforme	Natural infection	8	20.7	18.4
A. tamarii	Inoculation	1	18.7	19.8
A. niger	do	2	19.1	20.1
P. oxalicum	do	2	19.0	20.8
P. expansum	do	1	20.1	20.8
D. zeae	do	2	21.0	21.9
G. zeae	do	3	21.2	22.3
N. sphaerica	do	3	21.1	22.5
Cephalosporium acremonium	Natural infection	1	22.1	23.4

Aspergillus versicolor, *A. wentii*, and *A. ochraceus* developed at moistures only slightly higher than *A. glaucus* (table 3). *A. flavus* was not observed until a moisture content of 18.3 was reached, although it has been reported (*20*) as occurring in corn meal at a moisture as low as 16 percent. *A. niger* was found on grain with moistures of 18.3 percent and upward, while *A. tamarii*, observed in one test only, occurred at 19.8 percent. All of these species were observed growing naturally on unsterilized corn used in the experiments, but the lower limit for growth could not always be determined in that way. Some of the fungi that were observed were, therefore, isolated in pure culture and inoculations were made on surface-sterilized corn. No effort was made to determine the number and identification of all the fungi occurring naturally at the different moistures. In small grains *A. niger*, *A. flavus*, and *A. fumigatus* have been reported growing at 18 percent moisture (*6*). In another report (*14*) *A. glaucus* and *A. albus* were mentioned, the latter developing somewhat later and apparently requiring more moisture than *A. glaucus*.

Fungus growth at the lowest moistures occurred at the tip ends of the kernels where they had been attached to the cobs and at places where the seed coat was broken. At slightly higher moistures by close inspection a sparse amount of mycelium could be seen trailing through the spaces between the kernels, as shown in figure 3, *C*, and the formation of conidial heads was not limited to the places just mentioned. Different species showed some differences in growth habits.

PENICILLIUM SPECIES

Penicillium was found growing on the tips of corn kernels at moistures as low as 16.3 percent. By inoculating with some known cultures, the arrangement of species with respect to moisture requirement in ascending order was *P. notatum*,[4] *P. viridicatum* (in one of its forms),[4] *P. palitans*,[4] *P. oxalicum*,[5] and *P. expansum*.[6] The first three named were isolated from "blue eye" corn about which more is given below. *P. oxalicum* occurs commonly on corn ears before harvest when kernels have been mechanically injured by corn earworms or birds.

Penicillium expansum was included because McHargue (*14*) reported it as making more prolific growth than any of the molds observed in his corn-storage experiments. However, the culture of *P. expansum* used by the writer made the least vigorous growth on corn of any of the penicillia used. This difference in results may very likely be explained by differences in physiologic behavior of different strains of *P. expansum*. There is the question also whether *P. expansum* is considered in the broad sense in which some forms may be difficult to distinguish from some members of the *P. viridicatum* series, or whether the species is limited to that organism which, in addition to answering the morphological description, causes rot of apple.

A special study was made of the condition known to the grain trade as "blue eye" (fig. 4). It is caused by the growth of blue penicillia between the germ and the seed coat. Through the courtesy of W. B. Combs and H. P. English, of the Bureau of Agricultural Economics United States Department of Agriculture, samples of blue eye were obtained from the markets at Chicago, Milwaukee, Minneapolis, Cedar Rapids, Peoria, Toledo, and Nashville. Representative kernels but with unbroken seed coats were selected from each lot. They were surface-sterilized with a chlorinated lime solution, and the seed coat covering the germ was then opened and a transfer of the fungus spores made to sterile water. This was followed by the pouring of agar dilution plates. Sometimes the colonies obtained in the plates from a transfer from a single kernel appeared to be all alike; sometimes two different kinds of colonies developed in abundance, and occasionally there were three different kinds. All the isolations were penicillia. To make more sure of their purity, a needle transfer was made from the one or from each of the several kinds of colonies isolated from each kernel and a second set of dilution plates was poured.

After spending a whole winter season isolating, purifying, culturing and classifying these penicillia, and studying the keys and descriptions (*19*), the writer felt that he could not definitely name any of them

[4] Identified by Thom.

[5] Received from Helen Johann, of the Bureau of Plant Industry, U. S. Department of Agriculture, who had it identified by Thom.

[6] Received from H. W. Anderson, University of Illinois, who isolated it from a rotting apple.

unless he were to make a long-time study of the whole *Penicillium* group and secure an abundance of authentic specimens. As this was not the main purpose of the investigation, such a study was not attempted. Many of the isolations could be classified into three large groups within each of which there was a great deal of similarity. A fourth group was composed of various types which did not classify into the first three. No further work was done with the last group. Representative samples of the first three groups sent to Dr. Charles Thom in the spring of 1935 and again in 1936 were identified, while others were said to be mixtures. From the results of these identifications it appeared that for the most part one group represented members of the *P. chrysogenum* series, one group was *P. palitans*, a

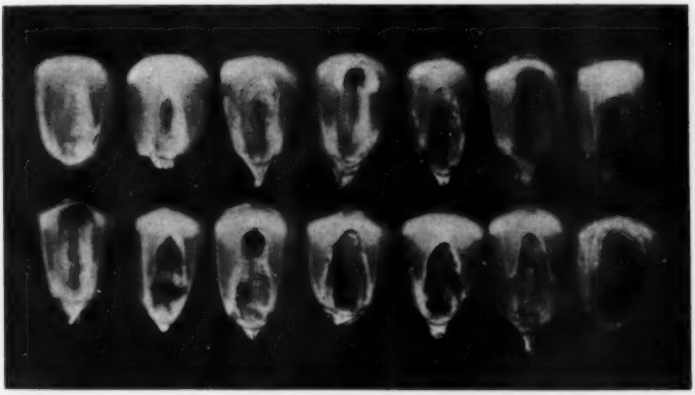

FIGURE 4.—Blue eye of corn caused by a growth of *Penicillium* over the germ and beneath the seed coat on grain with moistures of 18.5 to 24.0 percent. These specimens were received from Federal grain inspectors. Very little blue eye has been observed above the moistures given, for at higher moistures other molds usually predominated over the penicillia.

member of the *P. viridicatum* series, and the other group was composed of members of the "Fasciculata," ranging, according to Thom, "from *P. cyclopium* through the *viridicatum* series."

The lower moisture limits for the development of blue eye was determined by (1) observing its natural development from surface-borne spores, and (2) by inoculating surface-sterilized corn with *Penicillium notatum*, *P. palitans*, and with three different cultures belonging to the third group mentioned above. Corn not inoculated did not always develop blue eye even though moisture was adequate, but at times abundant blue eye developed. In the inoculated series *P. notatum* produced blue eye in 2 percent of the kernels at 16.7 percent moisture, in 10 percent of the kernels at 17.7 percent moisture, and in about 60 percent of the kernels at 19.0 percent moisture. *P. palitans* produced 10 percent of blue eye kernels at 19.5 percent moisture and about 50 percent at 21.2 percent moisture. The general appearance of the blue eye from the two inoculations was practically alike, being blue in color. The three unnamed species caused blue eye at about the same moistures as *P. palitans*, and one of them produced blue eye identical in appearance with it. Of the other two, one produced a grayish-blue color of the germ area, and the other produced a some-

what reddish or purple color. When kernels of the latter kind were placed in an alkaline solution the color became a bright blue. *P. oxalicum* and *P. expansum*, although growing and fruiting over the surfaces of the kernels, did not cause the typical blue eye condition.

At the lowest moistures, blue eye appeared mostly as a narrow stripe over the germ, as illustrated in figure 4, top row, second kernel from the left. This condition, called "hair line blue eye" by grain inspectors, in some forms is easily confused with the genetic condition known as purple plumule (*8*). When a kernel is cut crosswise through the plumule, however, the genetic purpling is seen to be in the tissue of the plumule, while the fruiting heads of penicillia causing the color of blue eye are located between the germ and the seed coat. With somewhat higher moistures blue eye becomes more general over the surface of the germ. At moistures above 23 to 26 percent, depending no doubt largely on what kind of fungi the grain is carrying, blue eye is likely to become obliterated by the growth of other organisms. Sometimes it is covered over by various aspergilli even at moistures below 23 percent. In natural, unsterilized stored shelled corn with moistures of 17 to 23 percent, sometimes the penicillia, sometimes the aspergilli, predominate.

FUSARIUM MONILIFORME

That there are wide differences in culture characters of *Fusarium moniliforme* is well known, and marked differences in pathogenicity to corn have been reported (*13*, *21*). Differences were also found with respect to moisture requirements. Because of the variability in the results obtained, more tests were made with this fungus than with any other. Only naturally infected seed was used. Infection caused by *F. moniliforme* is the commonest of all internal seed infections in corn, and a great many kernels of each lot used in these particular tests carried this fungus. The term "pure culture" as applied to *F. moniliforme* in table 3 means only that no other kinds of fungi were present; most certainly there were a considerable number of strains of *F. moniliforme* in each lot of corn used and the growth at the lower moisture limit was determined by the particular strain that had tbe lowest moisture requirement. The 22 different experiments were conducted with 18 different lots of corn over a period of 8 years. Corn internally infected with other organisms of course was avoided in these tests.

In surface-sterilized grain growth of *Fusarium moniliforme* was seen at 18.4 percent moisture in one lot, while in another lot no growth occurred at 20.7 but growth did occur at 22.1 percent. In grain not surface-sterilized the end point for growth was more difficult to determine because some aspergilli and penicillia grow luxuriantly at moistures where growth of *F. moniliforme* is very feeble. However, by using a microscope and pouring dilution plates from needle-point transfers from suspected places, the presence of *F. moniliforme* could be definitely verified. In some of these tests the fungus was found to grow at moistures as low as 18.8 percent, while in others the grain was free from growth up to or above 21.6 percent. There seems to be no doubt that the variability of different strains of *F. moniliforme* was the principal cause of some of these differences. Furthermore, the strains used or observed probably do not represent the whole range,

for, as already pointed out, measurements were concerned entirely with those strains in each lot of corn that had the lowest moisture requirement.

Above 21 to 24 percent moisture *Fusarium moniliforme* grew vigorously, completely enveloping the grain with a powdery pink mass and with mycelium crisscrossing the spaces between the kernels. Frequently many of the germs became rotted and turned a deep reddish-purple color. In mixed cultures it sometimes was difficult to distinguish this discoloration from that produced by certain penicillia, although the usual type of blue eye produced by penicillia is decidedly bluish in color.

Commercial lots of corn examined over a period of years have always shown 10 percent or more infection from *Fusarium moniliforme*. Many infected kernels look normal and healthy. Two methods of testing have been used. (1) a tray germinator in which the corn is tested as received, and (2) plating the surface-sterilized grain on potato-dextrose agar. The percentage of infection has always been much higher by the first method than by the second, indicating that much of the infection was superficial enough to be killed by the disinfectant used. It is evident that most if not all lots of corn from the Corn Belt contain enough *F. moniliforme* infection to cause serious rot above 23 percent moisture if the temperature and oxygen supply are suitable. At this moisture *F. moniforme* will usually compete well and frequently will predominate over the aspergilli and penicillia, but if *Diplodia zeae* is present in sufficient quantity it, in turn, will predominate over *F. moniliforme*.

DIPLODIA ZEAE AND OTHER FUNGI

The moisture limits for growth of *Diplodia zeae*, *Gibberella zeae*, and *Nigrospora sphaerica* are very close together, as shown in table 3, and the general appearance of the three is very much the same (fig. 5). At 23.8 percent moisture *Diplodia* caused a dull dark discoloration of the germs and at slightly higher moistures all the kernels became tightly bound together by the white *Diplodia* mycelium. It grew aggressively and usually predominated over all other organisms.

Gibberella zeae had a pure white mycelium like *Diplodia* when grown at the lowest moisture limit, but at somewhat higher moistures the mycelium was pink in some areas and yellow and white in others, all colors occurring in the same basket of corn. At this moisture many of the kernels developed deep-red discolorations of the germs and other limited parts of the kernels. When the corn was not surface-sterilized, *G. zeae* did not usually predominate over aspergilli, penicillia, and *Fusarium moniliforme* until the moisture reached 26 percent and over. Above this moisture it became aggressive.

Nigrospora sphaerica grew as well as the two fungi just discussed when stored at corresponding moistures in pure culture (fig. 5). The mycelium was usually white but sometimes had a grayish appearance caused by the presence of spores. Some strains of *N. sphaerica* produced spores under the conditions of these experiments, while others produced none. The white parts of affected corn kernels turned a yellowish color at moistures allowing abundant growth of *Nigrospora*. When forced to compete with other fungi, this fungus grew poorly in stored corn containing less than 30 percent moisture. In three tests

with naturally infected grain, not surface-sterilized, and with moistures ranging up to 25 percent, *Nigrospora* could not be detected, whereas several other fungi were present in great abundance. In other similar tests some growth of *Nigrospora* could be detected by its characteristic spores at a moisture of 23.6 percent. The results obtained with *Nigrospora* infection in nonsterile seed are in agreement with some results reported by Durrell (*4*). Apparently this fungus is not ordinarily of importance as a cause of storage rot in corn even though it is present and there is sufficient moisture for its growth.

Cephalosporium acremonium infection in corn grain, while not ordinarily as frequent as that caused by *Fusarium moniliforme*, is never-

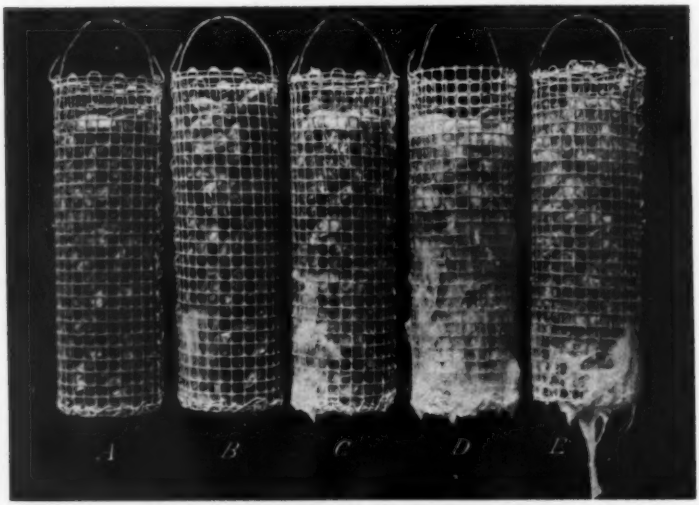

FIGURE 5.—*Nigrospora sphaerica* growing on corn in four baskets, *B* to *E*. No growth occurred in *A*. The storage period was 3 months at 70° F. with constant moisture of the grain as follows: *A*, 20.7 percent; *B*, 22.6; *C*, 23.4; *D*, 26.1; *E*, 29.2. At the same moisture content *Gibberella zeae* and *Diplodia zeae* present a similar appearance except that kernels infected by *G. zeae* turn a reddish color and portions of the mycelium may show yellowish to reddish hues, and those infected by *D. zeae*, which is a white mold, turn a brownish color and the mold usually is denser than that shown.

theless of very common occurrence. It is rarely found when plating surface-sterilized rot-damaged grain but is often observed when plating apparently sound grain. The explanation seems to be that *Cephalosporium* is not ordinarily a rot-producing organism. This belief was further strengthened by the storage experiments here reported. As is true with *F. moniliforme*, much of the *C. acremonium* infection is superficial enough so that thorough surface disinfection frees half or more of the kernels carrying this fungus. Nevertheless, by careful selection of corn ears, grain composites were prepared carrying 30 or more percent of *C. acremonium*, and no other fungus, after surface disinfection.

When corn grain was placed in the storage equipment without surface sterilization, other fungi developed in profusion at the higher moistures and *Cephalosporium* could not be detected by microscopic examination up to 21 percent moisture of the grain, which was the highest used. With surface-sterilized grain a pure growth of *Cephalo-*

sporium developed at suitable moistures. At 23.4 percent typical spore heads developed on sparse mycelium at the tip ends of the kernels and some of the kernels developed narrow longitudinal white streaks which were caused by the mycelium lifting the colorless seed coat away from the yellow endosperm. With higher moistures *Cephalosporium* growth became more abundant, but even at 27 percent moisture the growth was limited to the region at the tip ends of the kernels and there were no prominent indications of rotting after 3 months' storage.

DISCUSSION

A considerable number of tests were made with natural shelled corn, that is, unsterilized and uninoculated. Members of the *Aspergillus glaucus* group never failed to appear at 14.5 to 15.5 percent moisture in the grain and sometimes without apparent competition from other organisms. Some other aspergilli appeared at slightly higher percentages of moisture and some penicillia regularly appeared near 17 to 18 percent. They could be readily identified as penicillia among the aspergilli by the use of a microscope. A vertical illuminator which throws the light down on the object through condensers built around the objectives was found especially useful for distinguishing the various kinds of fungi. *A. niger*, a very easily recognized fungus, appeared in only about two-thirds of the tests at moistures suitable for its growth. Either this fungus was not universally present or slight amounts of it might have been inhibited by the growth of other organisms. This fungus, unlike some others, is so conspicuous that it would hardly be overlooked if present. *A. flavus* appeared in less than one-third of the tests.

In interpreting the data it must be borne in mind that in each test only a limited number of moisture constants were used with intervals of ½ to 2 percent of grain moisture ranging between them. Thus the exact moisture limit for fungus growth could not always be observed in any one experiment. Furthermore, experimental results did not always check closely. For instance, *Aspergillus wentii* was observed growing naturally on corn in two different tests made in two different seasons. In one case it was found at 15.1 percent but not at 14.3; in the other it was found at 16.2 but not at 15.2. Therefore, the data in table 3 show it as not present at 15.2 in one test, but as present at 15.1 in another. This fungus was not observed in 19 other tests with unsterilized seed, but this does not necessarily mean that it was absent, for it is not particularly conspicuous and can easily be overlooked when other fungi are abundant.

The variations in the results obtained in different tests can probably be accounted for by the fact that the corn used at different times came from different lots grown under different conditions. Moreover, different strains of the fungus species were present at different times. The results obtained with *Fusarium moniliforme* were especially variable, and this was charged primarily to the fungus itself as explained earlier under the discussion of that fungus.

Except in seed lots that had been carefully selected for freedom from *Fusarium moniliforme*, this fungus predominated above a moisture content of 23 percent provided *Diplodia zeae* was not present and provided the desired moisture was added at one time or the corn had not previously been dried. If dry corn was hung over solutions and the mois-

ture was taken up from the atmosphere the rise in moisture was slow (fig. 2) and aspergilli made heavy growth before *F. moniliforme* could get started. A fungus already in possession of the field may block the growth of another fungus which might have predominated if it had had an equal opportunity.

At a moisture suitable for vigorous growth of several fungi, the relative amount of inoculum of each kind present often determines which one will predominate. When different lots of unsterilized corn were dusted with several pure cultures of aspergilli and penicillia, respectively, the result in a number of cases was a growth of an almost pure culture of the fungus used for inoculation, almost as pure as though pure culture methods had been used.

When a *Penicillium* or *Aspergillus* species was found growing on grain at its lowest moisture limit it not only made weak growth but it also grew only on a minority of the kernels. At a slightly higher moisture the growth was not only more vigorous but it also occurred on a higher percentage of kernels. This appeared to be a combined effect of chance inoculation and actual difference in moisture content of individual kernels. It is also probable that some kernels possess resistance of a chemical nature to some extent (*11*). When kernels were thoroughly dusted with spores of one of these fungi before the experiment started, the fungus occurred on a much higher percentage of kernels even though it made weak growth for lack of water; still some kernels remained free from fungus growth. It has already been shown (table 2) that different kinds of corn may take up moisture at different rates or may come to equilibrium with their surrounding atmosphere with different percentages of moisture in the grain. This being true, individual kernels, especially of an open-pollinated variety, will no doubt behave differently in this respect also.

Actual damage to grain from rot was never observed at the lowest moisture limit for fungus growth. Damaged kernels of the kind classified as "commercial damage" by grain inspectors usually occurred to some extent with the various fungi tested at a moisture of about 1½ to 2 percent higher than the minimum moisture for growth. If the storage period had been longer, the critical moisture for development of damaged kernels might have been slightly lower. With temperatures higher than 70° F. the minimum moisture requirement and the margin for commercial damage both appear to be lowered slightly. When temperatures become sufficiently high heat damage takes place in addition to rot damage.

The oxygen requirements of the corn-rot fungi studied were very moderate. When 100 g of corn was suspended over a salt solution in a sealed bottle containing nearly 2 liters of air, growth with the various molds usually proceeded nearly as well as in an aerated bottle. A sour alcoholic odor developed in sealed bottles in which the moisture of the corn was over 16 percent. This odor was not observed in bottles aerated with only a fine caliber glass tube as mentioned in the discussion of methods. Rapid aeration with sterile air was used to carry off the chlorine when the corn had been surface-sterilized with chlorinated lime. If the sealed bottles had been filled full with grain, lack of aeration might have greatly retarded mold growth as Swanson found with wheat (*18*). In deep storage bins aeration also is exceedingly limited. Duvel (*5*) made observations in a 65-foot elevator bin filled with shelled corn at 17.0 to 18.8 percent moisture.

Storage was carried out during the late winter and spring months, the temperature being 36° to 39° F. when the experiment was started. In 5 weeks' time the mustiness had extended down to 7 feet below the surface but no lower. In 7 weeks no mustiness was observed at a 12-foot depth although there was an abundance of molds at the surface. When the bin was emptied after 9 weeks' storage the lower half still appeared to be free from significant mold growth.

SUMMARY

Shelled yellow dent corn was stored in atmospheres maintained at constant humidities of various degrees by placing it in wire baskets over salt solutions in closed containers. Storage was for a period of 3 months at a temperature of 70° F. Three methods of aeration were tried. The one supplying the least oxygen appeared adequate for growth of the fungi studied, but more liberal aeration was supplied for most of the tests.

Growth limits of a number of fungi as determined by moisture in grain was studied in two ways, in competition with surface-borne fungi, and in pure culture after surface-sterilizing the grain. The fungus to be studied was supplied by either selecting corn carrying the fungus as an internal infection or by inoculating the grain.

Different strains of corn showed distinct differences in the percentage of moisture in the grain when in equilibrium with a saturated atmosphere. There was good evidence also for believing that individual kernels showed variations in moisture content in experiments with open-pollinated corn. Thus one would not be able to predict accurately the moisture content of grain stored in an atmosphere with a known constant humidity.

Aspergillus glaucus grew at 14.3 percent moisture of grain, which was lower than could be utilized by any other fungus, but several other aspergilli appeared at only slightly higher moistures. *A. flavus* and *A. niger*, however, were not found below a moisture content of 18.3 percent.

Five species of penicillia were found to vary in their minimum moisture requirement from 15.6 to 20.8 percent. The blue color of the germ known to grain inspectors as "blue eye" was found to be caused by the growth of certain kinds of penicillia between the germ and the seed coat. *P. notatum* caused blue eye at a minimum moisture of 16.7 percent, while *P. palitans* required 19.5 percent, in these experiments. In each case somewhat more moisture was required for the extensive development of the blue-eye condition.

The minimum moisture requirement for *Fusarium moniliforme* ranged from 18.4 to 21.2 percent in tests in which competition from other organisms was excluded. A similar range was observed where competition was allowed. This was a wider variation than was observed with any other fungus species and demonstrated a significant variation in the moisture requirement of different strains of *F. moniliforme*.

Various aspergilli and penicillia grew well in mixed combinations in natural uninoculated seed. When the spores of a single species of these same fungi were dusted on the grain before a storage period was started, that particular fungus predominated more or less to the exclusion of all others provided moisture conditions were suitable.

Above 23 percent moisture *Fusarium moniliforme* competed well and often predominated over all other fungi except when *Diplodia zeae* was present. While in many cases one fungus tended to dominate another under growing conditions suitable for both, the relative abundance of inoculum of each one present was often an important factor in determining dominance.

Diplodia zeae, *Gibberella zeae*, and *Nigrospora sphaerica* all grew well on corn above 21.5 to 23 percent moisture content in the absence of competition from other organisms. When forced to compete, *D. zeae* was the most aggressive and *G. zeae* next. *N. sphaerica* was very weak.

For the production of commercial damage to the grain from rot by fungus growth an increase of 1½ to 2 percent in moisture over the minimum moisture requirement for growth was usually needed under the conditions of these experiments.

While *Cephalosporium acremonium* grew well on naturally infected corn under pure culture conditions, at moistures of 23.4 to 27 percent, it was not observed to cause commercial damage.

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SOME CORRELATIONS IN PLANT-TISSUE COMPOSITION, DECOMPOSITION PRODUCTS, AND EFFECT UPON CROP ROTATION WITH TOBACCO[1]

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INTRODUCTION

The concept of crop rotation has been developed largely through long experience with increased or decreased yields. In some degree the ideas on crop rotation have become a matter of tradition with little or no thought given to the reasons why one rotation is a success and another a failure. The underlying causes have long been suspected to be the action of residues of one crop upon the succeeding crop. The chemical and physical character of the soil is also undoubtedly affected by the selective nature of different plant membranes as receptors of ions and the element of time in the decomposition of residues.

Tobacco plants are especially sensitive to certain rotations. In farm practice, tobacco reacts most favorably when preceded by tobacco. Tobacco planted in rotation with ragweed and horseweed does not suffer any appreciable harm, but rotation with timothy or corn—in fact, with any of the grasses except redtop—has been found during certain seasons to be extremely unprofitable. Tobacco following corn, timothy, or clover is likely to suffer severely from brown root rot. In rotation with redtop, however, it seems to be less severely affected, but the trouble is not as perfectly eliminated as when either of the two weeds, ragweed or horseweed, is used, or when tobacco follows tobacco.

Examination of 45 different plants to learn the relative rate of decomposition of their tissues and the subsequent rate of ammonification and nitrification indicated that of all the seed plants used redtop decomposed most slowly. Then for this work followed timothy, corn, horseweed, and ragweed. Tobacco decomposed more rapidly than any of the other seed plants.

Related to this rate of decomposition is, of course, the carbon content of the soil and its reducing capacity. In two adjacent plots of the same soil types, the carbon content was higher in the one where corn preceded tobacco than in the other where tobacco grew for 2 consecutive years. The soil's greatest reducing capacity was well indicated by more nearly complete reduction of potassium bromate.

A field plot on which corn had been grown for 4 successive years was sterilized by passing an electric current through the soil to a depth of 6 inches. Tobacco planted in this soil developed without a trace of brown root rot. Plots on which corn had grown for 4 years were planted to *Chenopodium album* and *Amaranthus retroflexus* for 1 year. Tobacco grown on these plots the next year was much less affected by root rot than tobacco on a plot where corn had been grown for 5 years. Chemicals containing phenol as orthocresol, paracresol, and

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metacresol also improved the soil for tobacco both in pots and in the field. Another method that helped to prevent the rot was artificial drying of the soil previous to planting.

It seems that an organism, or a class of organisms, is specific for types of organic decomposition and that the products of their decomposition are different. Whether it is the activity of facultative parasites, obligate parasites, or saphrophytes, or their products which are harmful has not been discovered.

In the present work it was the author's desire to correlate the results obtained in the field with certain chemical structures and decomposition products arising from microbiological processes.

REVIEW OF LITERATURE

Differences in crop yield incident to different systems of rotation have been ascribed in part (1) to the toxic products arising from decomposition of specific types of plant tissues, and (2) to the ability of certain plants to remove from the soil some elements in too great abundance and thus leave to other plants little on which to subsist. The toxic compounds have been found to be relatively labile and readily changed by oxidation. Many of them contain an aldehyde group, either aliphatic or aromatic, or an unsaturated linking in their structure. Oxidation involves the conception of change of valence—loss of electrons. Schreiner and Shorey,[2] Breazeale,[3] and Collison [4] are of the opinion that these abnormal compounds influence the subsequent crop.

Experiments by Doran [5] and Eisenmenger [6] indicate that the infusions of certain plants cause retardation of growth of tobacco when applied to soil, and that the nitrogen fractions of different plant infusions are different. Nitrogen of tobacco attains an end point of ammonia or nitrate more rapidly than does the nitrogen of corn stover.

METHODS

The plants used for this experiment were corn (*Zea mays* L.); timothy (*Phleum pratense* L.); redtop (*Agrostis alba* L.); tobacco (*Nicotiana tabacum* L.); horseweed (*Erigeron canadensis* L.); and ragweed (*Ambrosia artemisiifolia* L.).

Each plant was dried, ground, and placed with soil in crocks of 1-gallon capacity. The total nitrogen for each plant species was determined, as was also the nitrogen of the soil. To each crock was added a different type of the ground plant tissue in such amount as to provide 11.09 g of nitrogen. This means, of course, that the amount of total organic matter was not the same in each crock. The crocks were kept in the greenhouse at approximately the same moisture content for 7 weeks. They were started at different times in order to permit the analyses to be done in sequence.

[2] SCHREINER, O., and SHOREY, E. C. THE ISOLATION OF HARMFUL ORGANIC SUBSTANCES IN SOILS. U. S. Bur. Soils Bull. 53, 53 pp., illus. 1909. See pp. 15–16.

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Plants at the usual stage of harvest maturity were chosen for the work. The ragweed and horseweed had ceased flowering and the lower leaves were dry.

At the end of 7 weeks each crock was removed, the material shaken with distilled water and filtered, first through coarse cloth and then through a Chamberland-Pasteur filter. A portion of the soil was dried at 110° C. and the moisture determined. The total nitrogen content of the soil was determined in this phase of the work by the regular Kjeldahl method.

In determining the total nitrogen of the extract, an aliquot portion, 50 cc, was made acid with sulphuric acid containing salicylic acid and taken down to dryness on a water bath. The modified method of Ranker was then followed.

For ammonia nitrogen, 100 cc of the soil extract was made alkaline with calcium oxide and the ammonia distilled into a standard acid (0.02 N) by steam distillation.

Protein nitrogen was determined by precipitation with ferric oxide hydrosol while hot, and filtered. The nitrogen in the residue was determined by the regular Kjeldahl method of digestion and distillation.

Amides were determined as follows: From 200 cc of protein filtrate, the ammonia nitrogen was driven off by the use of steam distillation and calcium oxide. After the remaining solution had cooled, sufficient sulphuric acid was added to make it equal to a 5-percent solution. The filtrate was hydrolyzed for 2 hours by boiling, a reflux condenser being used. At the end of the hydrolysis period, the solution of ammonia was driven over by steam distillation into standard acid (0.02 N).

Humin nitrogen was determined by taking the residual material in the flask from which the hydrolyzed amide, or ammonia, had been distilled, filtering it while hot, and treating the residue by the Kjeldahl method used in the protein determinations.

After ammonia nitrogen had been removed from a portion of the soil filtrate, the alpha amino acid nitrogen was determined by using the Van Slyke apparatus.

The nitrate nitrogen content was found by determining the total nitrogen after reducing the nitrates in the acid solution with metallic iron and comparing this with a similar portion which had not been reduced. The difference represents the nitrogen derived from nitrates.

In a separate experiment, the carbon of each of these plant tissues was determined by an electrical combustion method.

All analyses represent triplicate trials, and most of them are the average of six trials.

RESULTS AND DISCUSSION

After the materials had been subjected to decomposing agencies, the grasses appeared to be least decomposed, in some instances not even losing their original configuration. This was especially true of redtop, which seemed to be very slow in becoming an integral part of the soil and with it assuming a homogenous mass. Tobacco, on the other hand, to all appearances decomposed most rapidly. None of the fibers maintained their original identity. This was true of horseweed and ragweed also, but to a slightly less degree.

The carbon content of the various soils at the expiration of the period allowed (7 weeks) was different, the degree of difference depending

upon the relative slowness of decomposition. It is obvious, however, that though all soils contained the same amount of nitrogen at the beginning, not all contained the same amount of carbon. It is not axiomatic that plants with a higher nitrogen content decompose more rapidly. The amount of lignin and pentosans or products ensuing with maturity exert an influence toward preservation of the included tissue. At the end of 7 weeks, the soils to which the different vegetable tissues had been added contained the following percentages of carbon: Timothy, 2.97; redtop, 5.33; corn stover, 5.21; ragweed, 2.55; tobacco, 2.56; and horseweed, 2.48.

The grasses seem to decompose more slowly than the dicotyledonous plants used. Incidentally, it may be stated that the tissue of nonvascular land plants of the lower order decompose more slowly than do dicotyledonous plants, and that the higher order of plants, the monocotyledons (expecially the Gramineae), with few exceptions decompose more slowly than do the dicotyledonous plants. This applies to herbaceous tissue. It seems probable that grasses which can tolerate the greater soil acidity are less easily decomposed than those which require a more basic medium. Also, those seed plants which can compete in a medium of rather high acidity contain relatively less calcium and more silicon.

The relative amounts of different forms of nitrogen in a specific type of plant tissue may indicate the degree to which the materials have been built up; in other words, to what stage anabolism has progressed. When the tissues are in the process of breaking down, the end products, ammonia (under anaerobic conditions) or nitrates (under aerobic conditions), in greater abundance would indicate a greater rate of retrogression. The large percentage of proteins would indicate slow nitrification by agencies involved in the process. Larger amounts of amino acids and amides would indicate not a slow decomposition rate, but slow progress in reverting to end products—ammonia and nitrates.

The stages of decomposition in part are indicated in the data shown in table 1.

TABLE 1.—*Percentages of nitrogen in various forms found in soil extracts derived from mixing different types of plant tissue with soil and allowing them to decompose*

[Results computed on dry-weight basis]

Plant	Total of soil and plant residues	Total of soil extract	Protein	Amide	Alpha-amino acid	Ammonia	Nitrate	Humin
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Timothy	0.2523	0.0222	0.0167	0	0.0032	0.0026	0	0
Redtop	.2790	.0534	.0023	.0010	.0024	.0078	.0266	.0000
Corn stover	.2545	.0445	.0238	.0000	.0046	.0093	.0009	.0028
Ragweed	.2384	.0214	.0051	.0010	.0062	.0012	.0094	.0000
Horseweed	.2008	.0117	.0042	.0007	.0042	.0006	.0029	.0010
Tobacco	.2008	.0221	.0028	.0010	.0073	.0005	.0086	.0009

The protein values of timothy and corn are relatively high, whereas that of redtop is low. This is not in accord with the appearance of the materials when removed. But it has been observed that in rotation with tobacco redtop does not exert the same deleterious effect as timothy and corn.

The total soluble nitrogen of the extracts is higher for the grasses, though the values for timothy and tobacco are not materially different. This would imply that less denitrification took place in the grass-soil media than in the other media. Also, it is possible that the more soluble forms of nitrogen were less evenly distributed in the crock than before the more soluble forms appeared.

The amides, though exceedingly low in all media, had a tendency to appear among the plants that are less harmful in a tobacco rotation, that is, tobacco, ragweed, horseweed, and redtop.

The amino acid results showed no correlation between the plants that generally are regarded as good and those generally regarded as bad in a tobacco rotation.

Nitrate nitrogen formation in the variously treated soils indicated clearly that nitrates are not so readily formed in soils containing residues of timothy and corn as in soils containing tissues of plants regarded as favorable in tobacco rotation.

In respect to nitrogen decomposition products, corn and timothy tissues decomposed in soil have some properties in common, and in other respects redtop behaves like them. All three are highest in total soluble nitrogen, timothy and corn are highest in protein nitrogen; timothy, corn, and redtop are relatively higher in amides and ammonia; and ragweed, horseweed, and tobacco are high in nitrates from soil extract. Nitrates in excess of others would suggest less reductase activity.

It would seem that corn and timothy are in one class, ragweed, horseweed, and tobacco in another; and that redtop has some characteristics of both classes.

Some other properties of plant tissues were determined in another experiment in order to extend the correlations. Table 2 indicates some of these relationships.

TABLE 2.—*Percentages of organic constituents of plants as indicating the relative degree of ammonification and nitrification of organic nitrogen during 63 days and growth-promoting capacity of plant-tissue decomposition products when 0.41 g of nitrogen from different plants was added to soil*

[The crocks containing the soil were of 8 pounds capacity]

Plant	Total nitrogen	Total carbon	C/N ratio	Pentosans	Lignin	Average weekly total ammonia and nitrate nitrogen in soil	Dry weight of barley [1]
	Percent	*Percent*		*Percent*	*Percent*	*P. p. m.*	*Grains*
Timothy	0.86	50.0	58	18.7	13.9	4	2.1
Redtop	1.02	45.9	45	27.2	9.8	2	1.4
Corn stover	.86	47.6	55	21.3	5.2	13	1.2
Ragweed	2.15	45.1	21	14.8	2.1	53	5.31
Tobacco	2.88	38.2	13	12.7	4.4	136	12.4

[1] Barley grown on soil to which 0.41 g of nitrogen from different plants was added.

Data for horseweed are not included, because no tissue of this plant was available.

Those plants that contain the lowest percentages of nitrogen, the highest percentages of carbon, have the highest carbon-nitrogen ratio,

a high percentage of pentosans, and the highest percentage of lignin, are the plants which, when placed in soil for a period of time, have the highest percentage of total soluble nitrogen, the lowest percentage of amides, and the highest percentage of ammonia nitrogen. Those plants that have the above-mentioned properties, also are characterized by low ammonification and nitrification values in soils, and low growth-promoting properties of barley (when all receive the same amount of nitrogen derived from different plants). Redtop resembles timothy and corn in all respects except in residual protein in the soils. Furthermore, the ammonia nitrates formed in the soil from redtop residues have a nitrification capacity similar to that of the plant residues not of the grass family—tobacco and ragweed.

In field practice it has been found satisfactory to follow ragweed by tobacco. On the college farm, redtop has proved more satisfactory than either corn or timothy for preceding tobacco, but never so satisfactory as tobacco preceding tobacco.

It is reasonable to suppose that, when roots and stubble of these various plants are permitted to decompose, the decomposition is slower, for the roots are invariably lower in nitrogen. It seems reasonable to suppose that high lignin and high pentosans would invite a general class of decomposing agencies different from tissues of simpler composition.

Little is known about the end products of decomposition of lignin, but the general type of methoxy compounds may arise, while starches and cellulose may, depending upon the reagents, be converted into monosaccharides, acids, and other products.

The fact that brown root rot of tobacco has been entirely eliminated from soils by sterilization with electric current, orthocresol, heating, or acetic acid and by drying, suggests that life processes are a factor in the malady, for none of these procedures would destroy the abnormal compounds associated with soil and specific plant tissue.

In field practice, we have other plants which do not lend themselves successfully to a place in tobacco rotations. Such plants as red clover and alfalfa, though not so pronounced in their harmful effects upon tobacco, have been found undesirable. These legumes when mixed with soil and allowed to decompose for several weeks convert the organic nitrogen to ammonia and nitrate nitrogen rather rapidly; alfalfa more readily than ragweed and less rapidly than tobacco. The organic nitrogen of red clover was converted into ammonia and nitrate nitrogen in soil at a rate only slightly less than that of ragweed. However, red clover and alfalfa contain a relatively high percentage of pentosans and lignin. A sample of red clover contained 15 percent of pentosans and 13 percent of lignic acid; a sample of alfalfa contained 17 percent of pentosans and 11 percent of lignic acid. These compared with 14.8 and 2.1, respectively, for ragweed and 12.7 and 4.4, respectively for tobacco, indicate at least a higher value of lignin in the two legumes.

The explanation is possibly found in the fact that the evolution of organic nitrogen to ammonia and nitrate nitrogen occurs in the initial state of decomposition, while one of the final steps in decomposition involves the conversion of lignin to organic derivatives found in soil, frequently referred to as "humus."

It is a well-known fact that organic materials containing high percentages of lignin may, when applied to soils low in nitrogen, seriously

diminish the growth of plants. Also, when the same organic matter is applied to soils too high in nitrogen, and conducive to abnormal growth and subsequent "lodging," it may exert a favorable influence. This influence is temporary, for when lignin is once decomposed, the result is more favorable than if none had been added to decompose. It has been suggested by observers that an old timothy sod is not as deleterious to a subsequent tobacco crop as one that has grown for only a few years. The inference is that the residues of previous years have to some degree overcome the results of newer decomposition activities.

Plant tissues in the early stages of development contain an appreciable amount of lignin, but when the plants are plowed under for green manures, the deleterious effects are not apparent. At this stage of growth, plants contain an abundant quantity of bound water and the rapidity of decomposition is augmented.

It would seem that what is apparently an unfavorable rotation for tobacco may be unfavorable for other crops, but to a less degree, for the tobacco plant is more sensitive than most plants to its environment.

Tissues of plants containing high percentages of lignin maintain their original structure, when killed, for a longer period than do those having a low content of lignin. Thus the stems and leaves of grasses and sedges have much the same configuration after winter has passed as when they were green, while tobacco, potato, and ragweed have little left to suggest their original appearance. In a general way we should be able to determine after a winter has passed those plants that would fit into a rotation system with tobacco.

SUMMARY

An attempt was made to find correlation in results relative to tobacco rotations obtained in the greenhouse, the laboratory, and the field.

From the laboratory test, an estimation was made of nitrogen fractions resulting from decomposition of different types of plant tissue in soil—corn, timothy, redtop, ragweed, horseweed, and tobacco. Also, the amounts of lignin, pentosans, carbon, and nitrogen and their ratio, rates of ammonification and nitration, and growth-promoting values were calculated when equal amounts of nitrogen from each of the different plants were added to soil.

Timothy and corn, which have been found unsatisfactory in tobacco rotation, showed a high percentage of total nigrogen, a high percentage of total soluble nitrogen, the highest percentage of protein, a low percentage of amides, a high percentage of ammonia, and the lowest percentage of nitrate nitrogen. Ragweed, horseweed, and tobacco showed lower percentages of each of these fractions except nitrates. The nitrates in this group were higher.

Redtop resembled the other grasses in the decomposition of organic nitrogen except protein and nitrates. It was relatively high in nitrates and low in protein.

The plants could be divided into two distinct groups. The first group, which comprised the grasses corn, timothy, and redtop, contained higher percentages of carbon, low percentages of nitrogen, a high carbon-nitrogen ratio, high pentosans, high lignic acid, low ammonification and nitrification, and low growth-promoting values.

The other group—ragweed and tobacco—showed lower carbon values, higher nitrogen values, a lower carbon-nitrogen ratio, lower pentosans, lower lignic acid, higher ammonification and nitrification, and higher growth-promoting values. In field practice, timothy and corn were found to be unsatisfactory for tobacco rotation. Ragweed, horseweed, and tobacco are desirable for rotation. Redtop is less desirable than tobacco and ragweed, but more desirable than timothy or corn.

It would seem that plants containing the higher values of lignin, pentosans, high carbon-nitrogen ratios, and a subsequent low tendency to protein decomposition in soil may be suspected of being undesirable for tobacco rotation.

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